


A nighttime photograph of the London skyline. The London Eye is illuminated in purple and blue on the left. In the center, the Houses of Parliament and Big Ben are brightly lit in yellow. The River Thames flows in the foreground, reflecting the city lights. The sky is dark, and the overall scene is a vibrant display of urban architecture at night.

# Metabolic regulation of immunity in chronic HBV infection

**Dr Laura Pallett**

Postdoctoral Research Associate – Prof. Mala Maini

A nighttime photograph of the London skyline. The London Eye is illuminated in purple and blue on the left. In the center, the Houses of Parliament and Big Ben are brightly lit in yellow. The River Thames flows in the foreground, reflecting the city lights. The sky is dark, and the overall scene is a vibrant display of urban architecture at night.

Metabolic regulation of immunity in  
chronic HBV infection by amino acid  
deprivation

**Dr Laura Pallett**

Postdoctoral Research Associate – Prof. Mala Maini

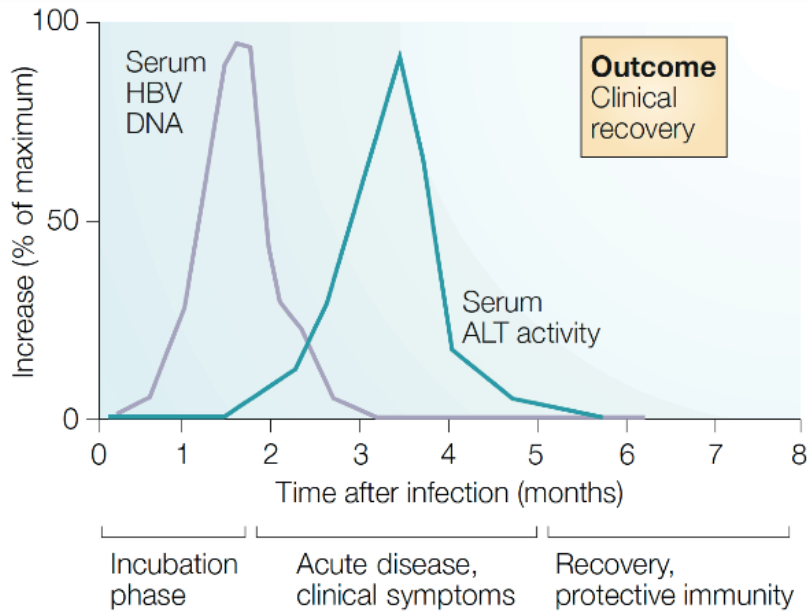
# Impact of Hepatitis B virus (HBV)

- Hepatotropic, non-cytopathic virus
- HBV establishes persistent liver infection in >300 million people worldwide
- Causes >600,000 deaths annually from associated liver disease
- Persistence is perpetuated by an inadequate virus-specific T cell response

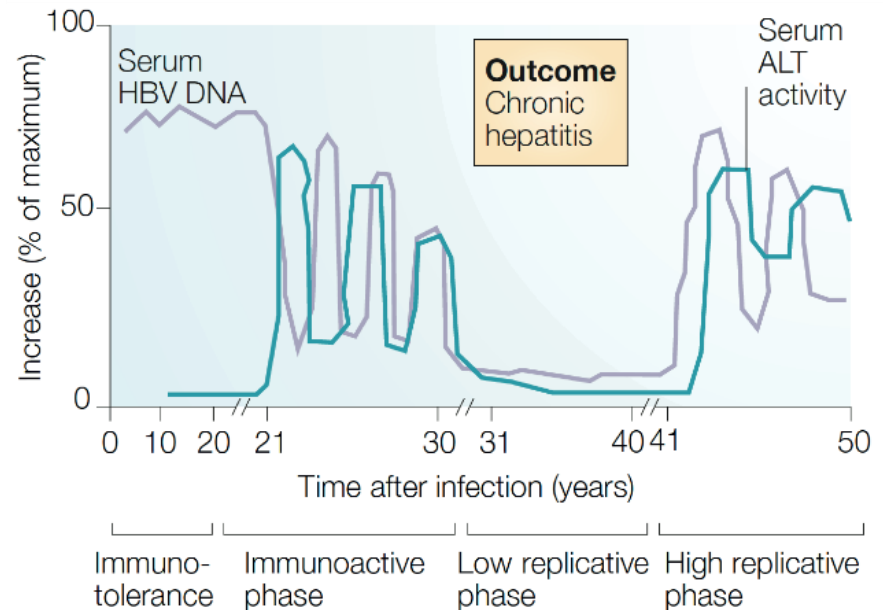
**Resultant liver disease is immune-mediated**

# Differential regulation of tissue damage in HBV infection

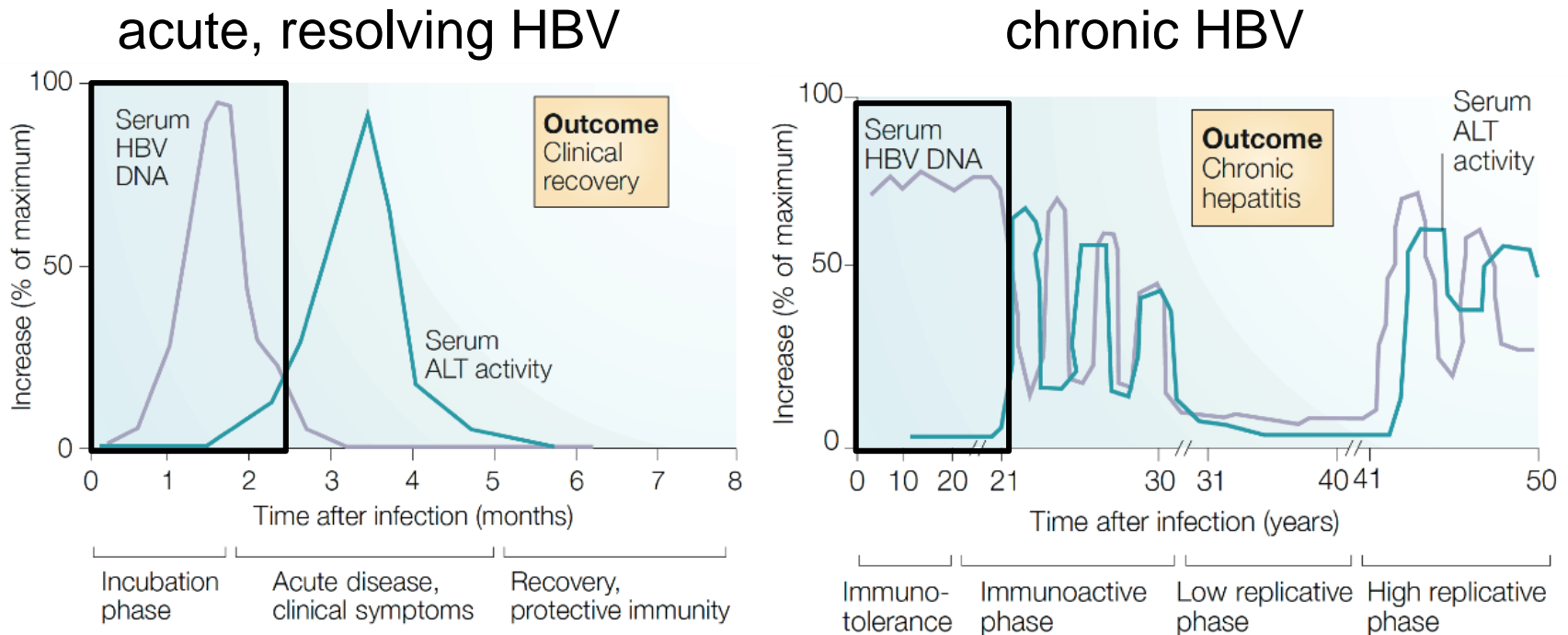
## acute, resolving HBV



## chronic HBV

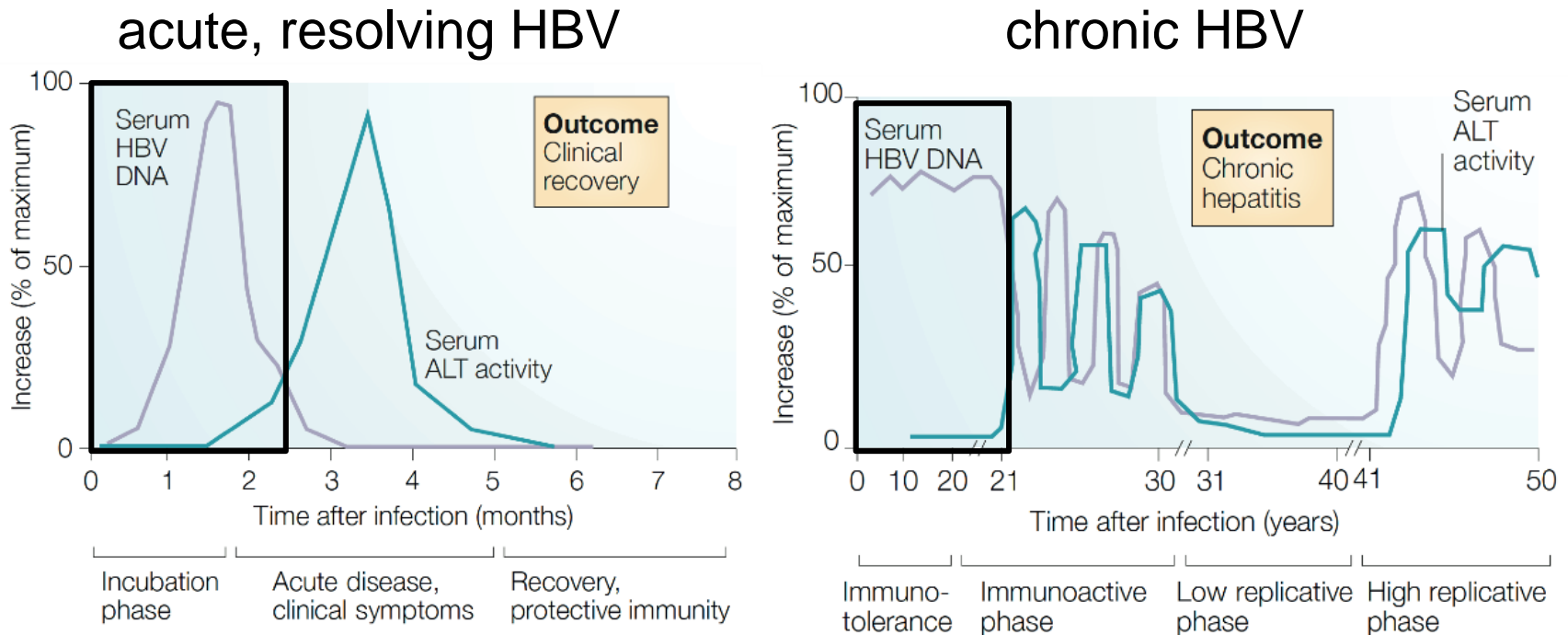


# Differential regulation of tissue damage in HBV infection



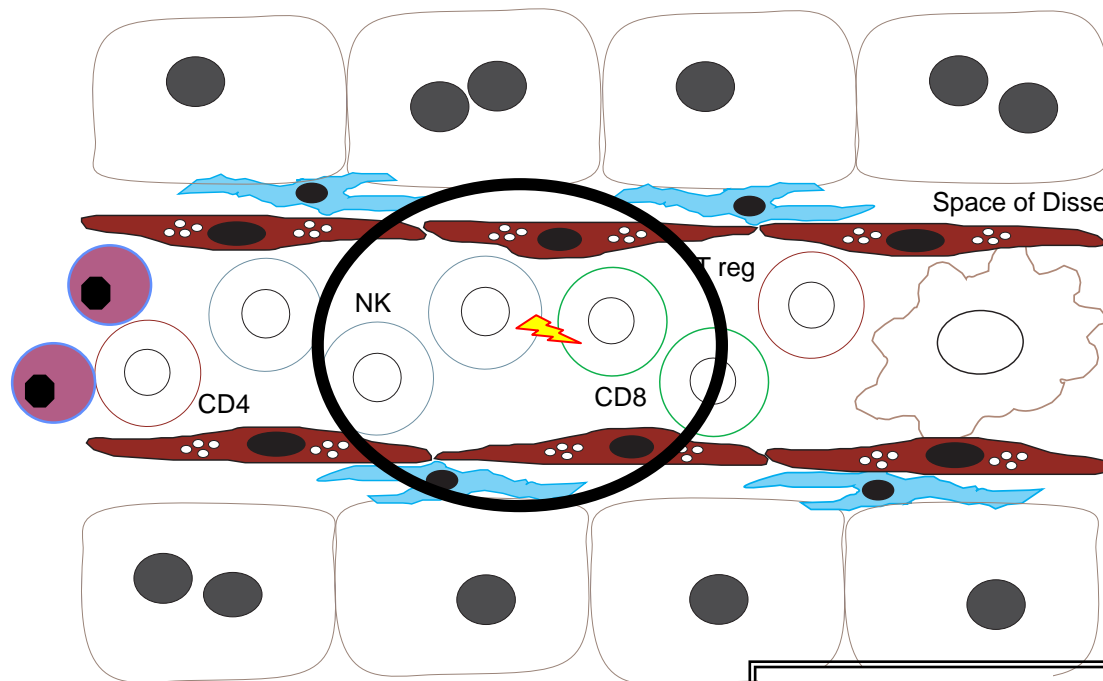


# Differential regulation of tissue damage in HBV infection



Paradigm for immunomodulation of organ damage - What mediates the differential regulation of liver immunopathology in different phases of HBV infection?

# Innate regulation of adaptive immunity in HBV infection



*Dimitra Peppas*



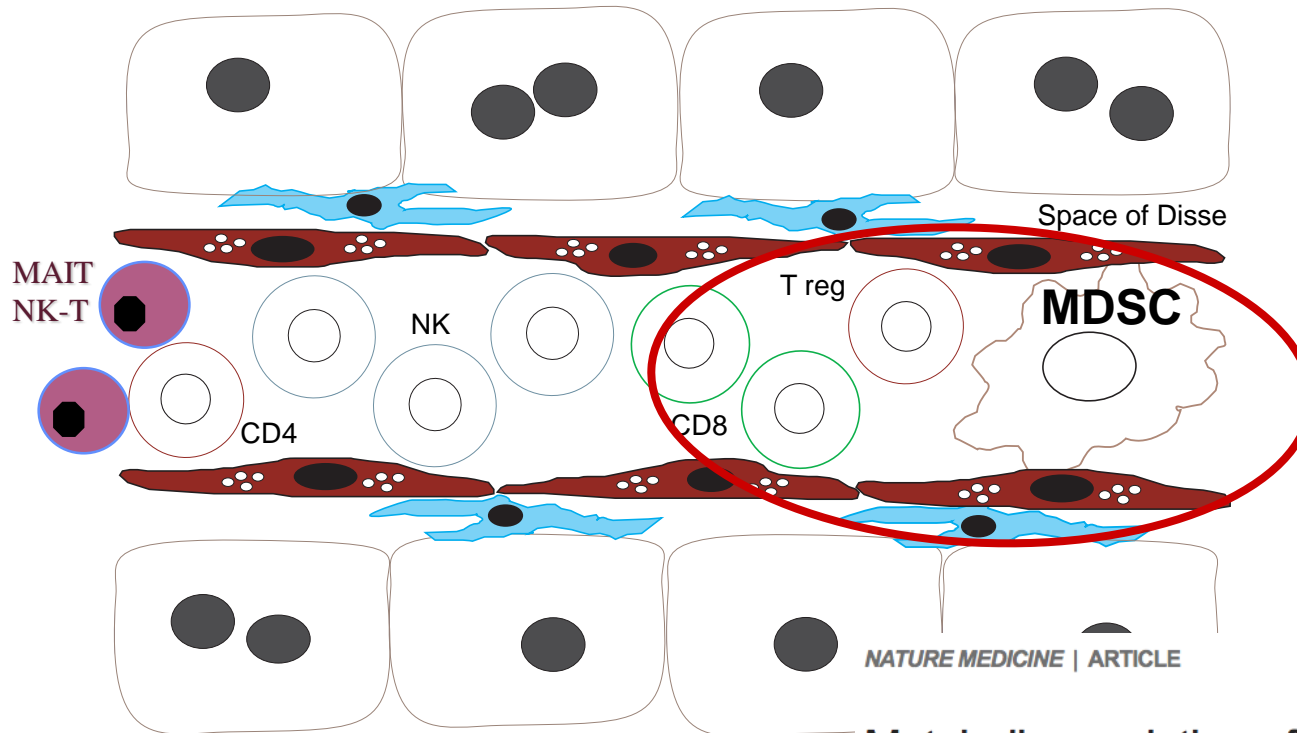
Rapid, contact-dependent NK cell killing of HBV-specific T cells

Published December 17, 2012

**JEM** Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion

Dimitra Peppas,<sup>1,2</sup> Upkar S. Gill,<sup>3</sup> Gary Reynolds,<sup>4</sup> Nicholas J.W. Easom,<sup>1</sup> Laura J. Pallett,<sup>1</sup> Anna Schurich,<sup>1</sup> Lorenzo Micco,<sup>1</sup> Gaia Nebbia,<sup>1</sup> Harsimran D. Singh,<sup>1</sup> David H. Adams,<sup>4</sup> Patrick T.F. Kennedy,<sup>3</sup> and Mala K. Maini<sup>1</sup>

# Role for myeloid-derived suppressor cells in regulating liver immunopathology

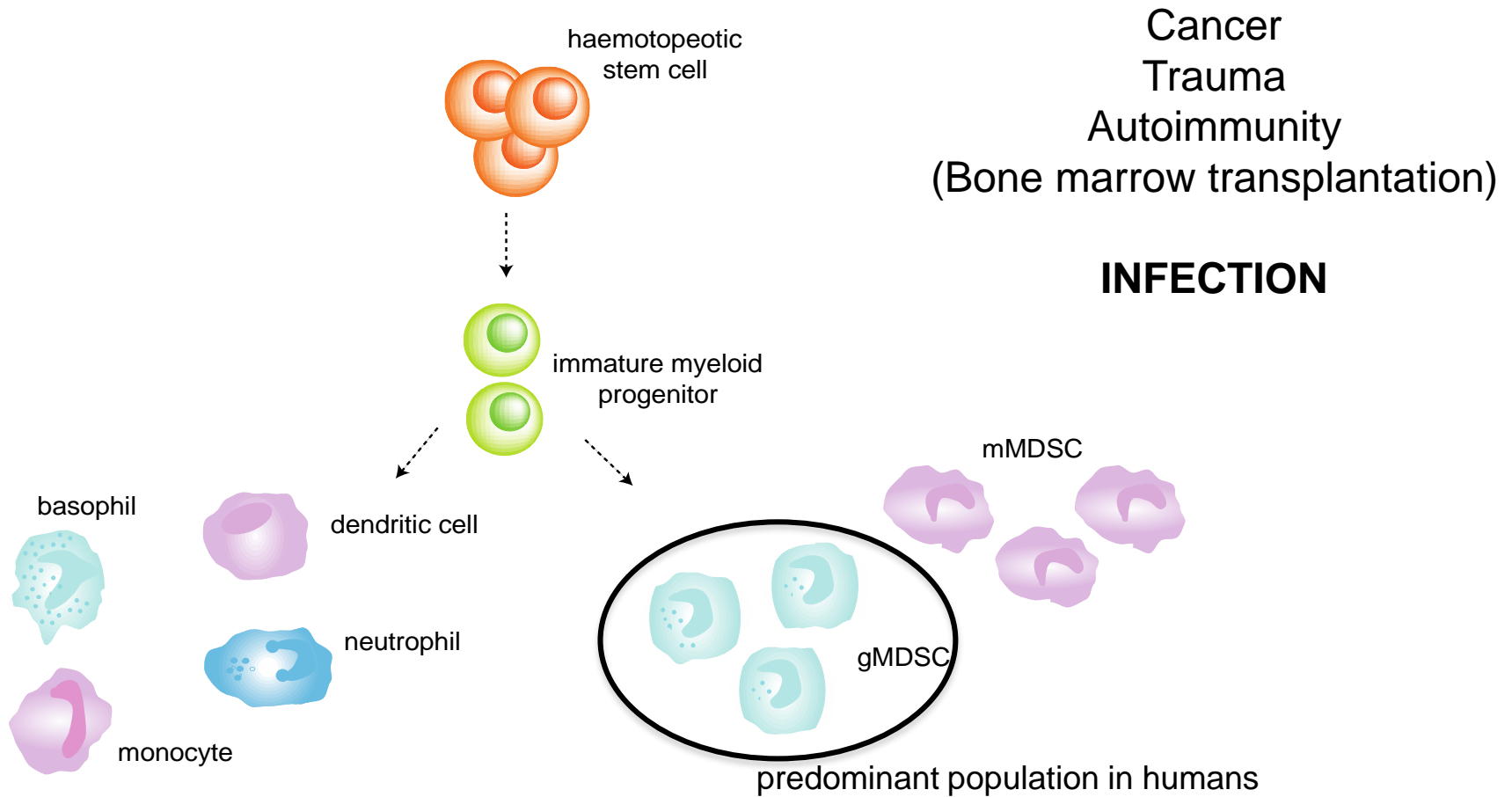


Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells

Laura J Pallett, Upkar S Gill, Alberto Quaglia, Linda V Sinclair, Maria Jover-Cobos, Anna Schurich, Kasha P Singh, Niclas Thomas, Abhishek Das, Antony Chen, Giuseppe Fusai, Antonio Bertoletti, Doreen A Cantrell, Patrick T Kennedy, Nathan A Davies, Muzlifah Haniffa & Mala K Maini

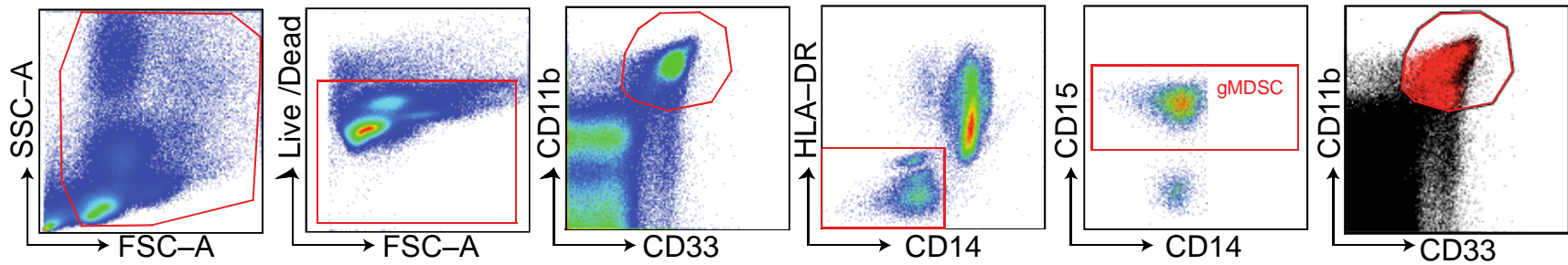


# What are granulocytic myeloid-derived suppressor cells?

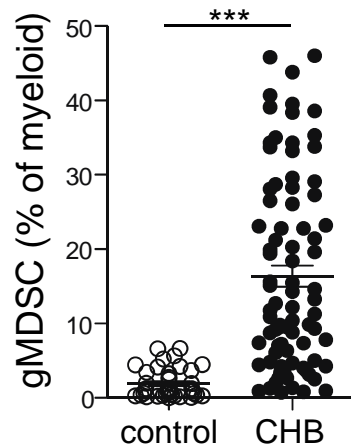


# gMDSC expand in hepatotropic viral infection (HBV)

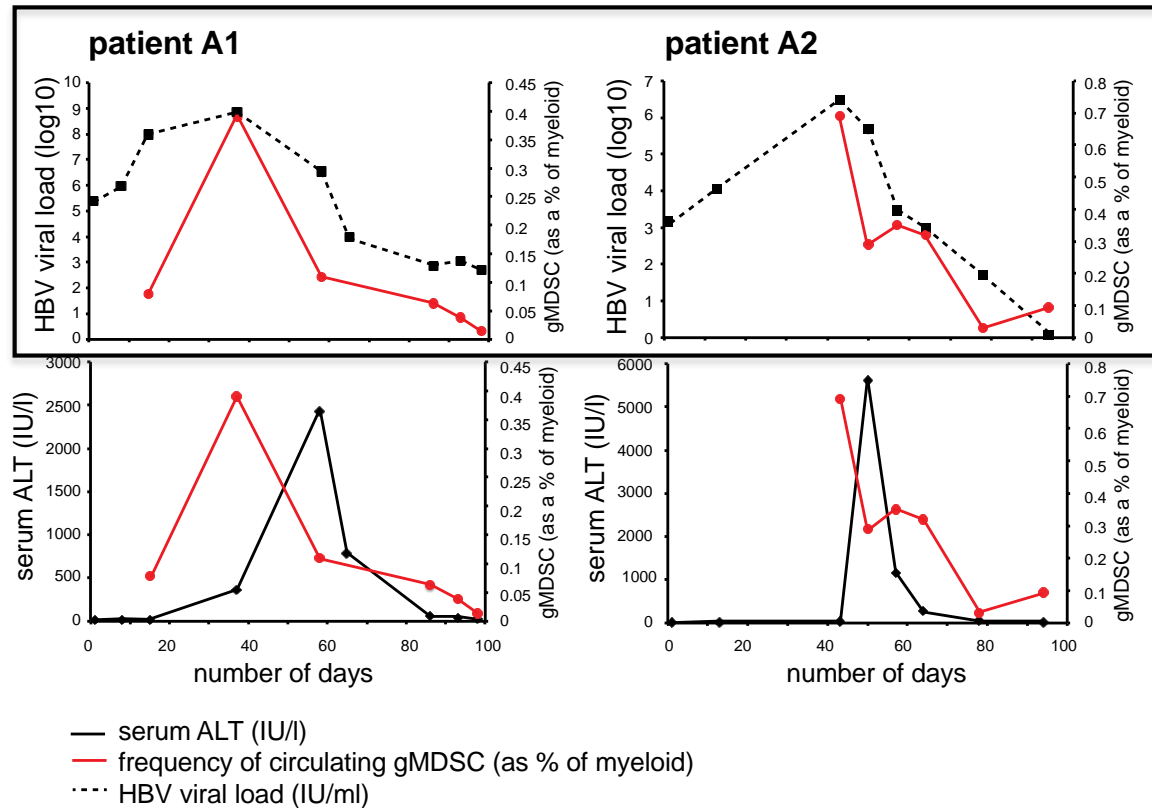
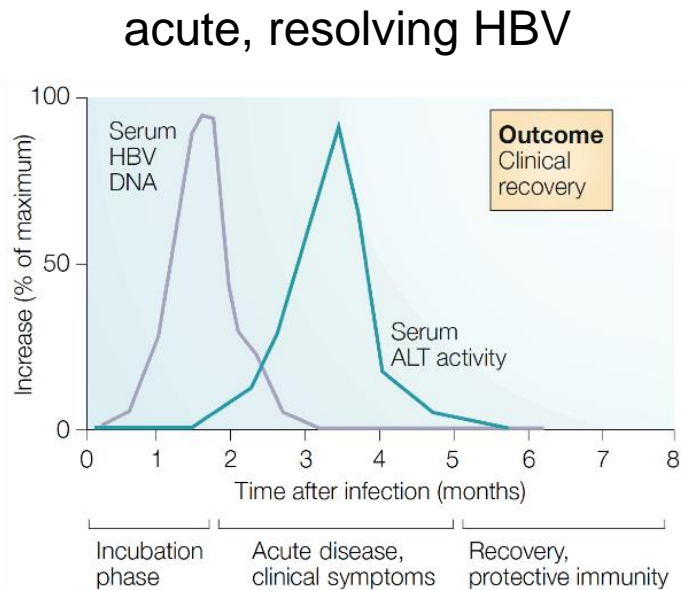
## 11 colour flow cytometry - peripheral blood



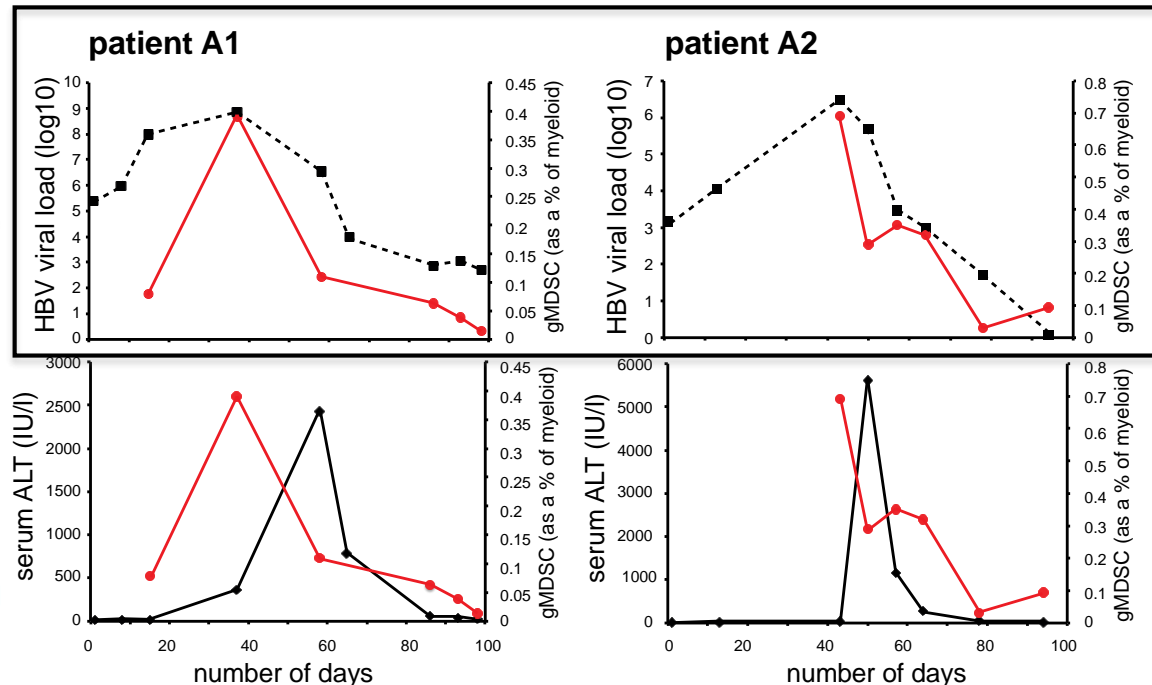
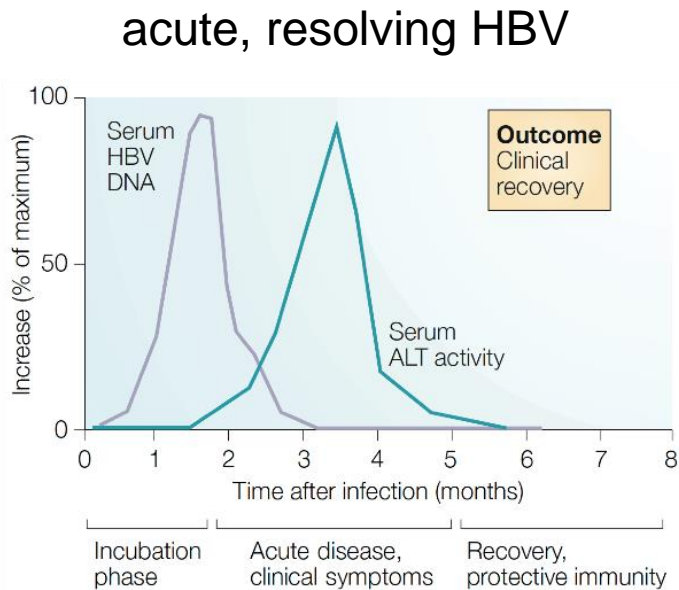
## all chronic HBV patients



# gMDSC transiently expand in acute HBV in parallel with viraemia



# gMDSC transiently expand in acute HBV in parallel with viraemia



— serum ALT (IU/l)  
 — frequency of circulating gMDSC (as % of myeloid)  
 ···· HBV viral load (IU/ml)

Immunity  
Article

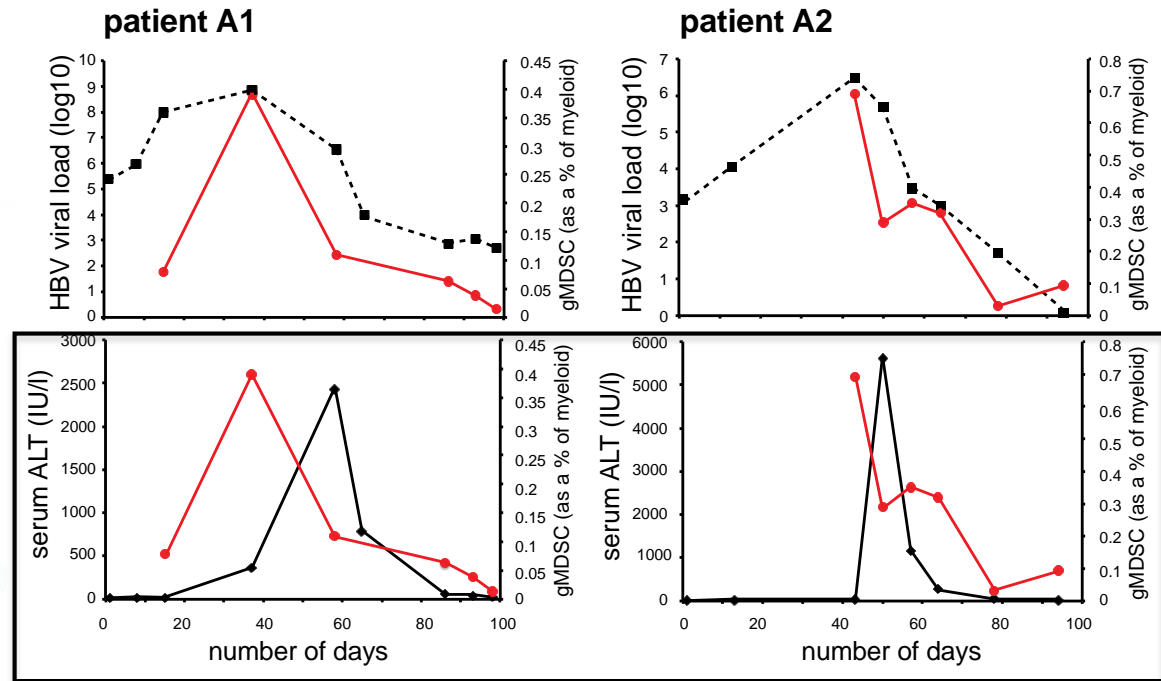
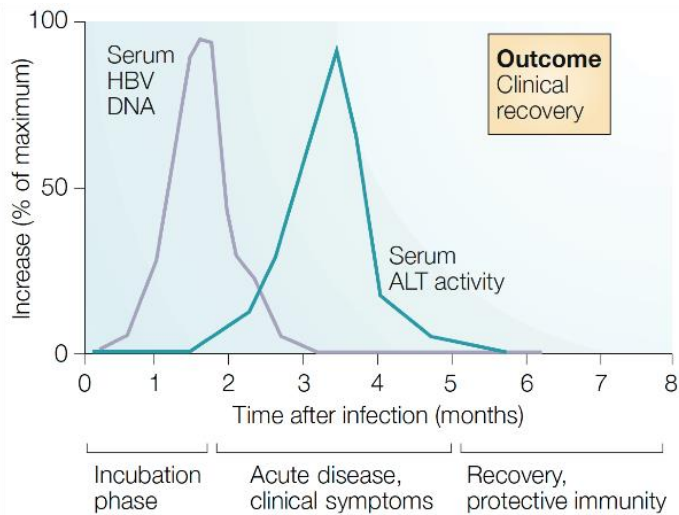


**Chronic but Not Acute Virus Infection Induces Sustained Expansion of Myeloid Suppressor Cell Numbers that Inhibit Viral-Specific T Cell Immunity**

Brian A. Norris,<sup>1,2,3</sup> Luke S. Uebelhoer,<sup>1,2,3</sup> Heider I. Nakaya,<sup>1,2,3</sup> Aryn A. Price,<sup>1,2,3</sup> Arash Grakoui,<sup>1,2,3</sup> and Bali Pulendran<sup>1,2,3\*</sup>

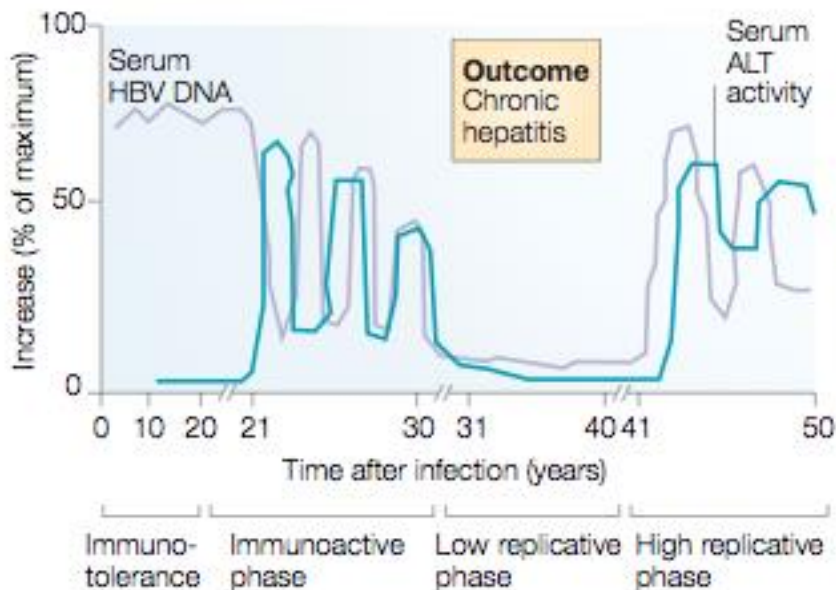
# ...declining at the onset of liver-specific inflammation

## acute, resolving HBV



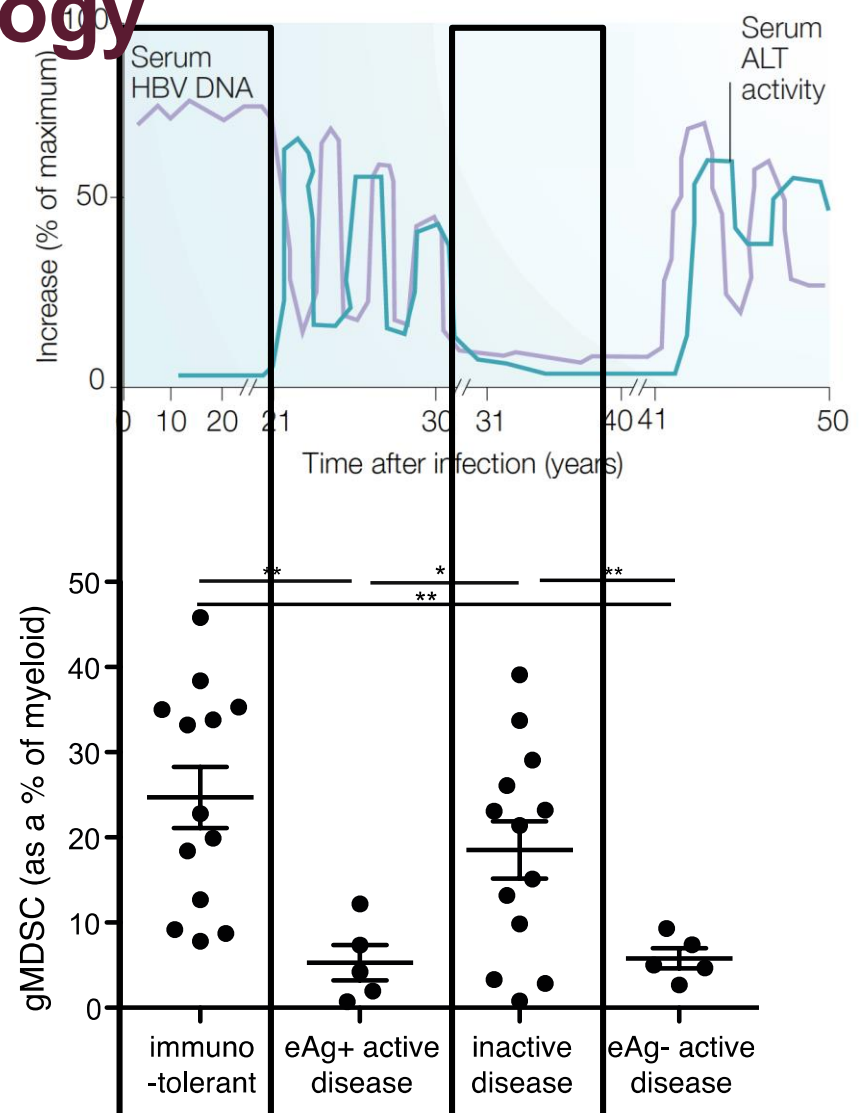
- serum ALT (IU/l)
- frequency of circulating gMDSC (as a % of myeloid)
- HBV viral load (IU/ml)

# gMDSC expand in patients replicating HBV without immunopathology

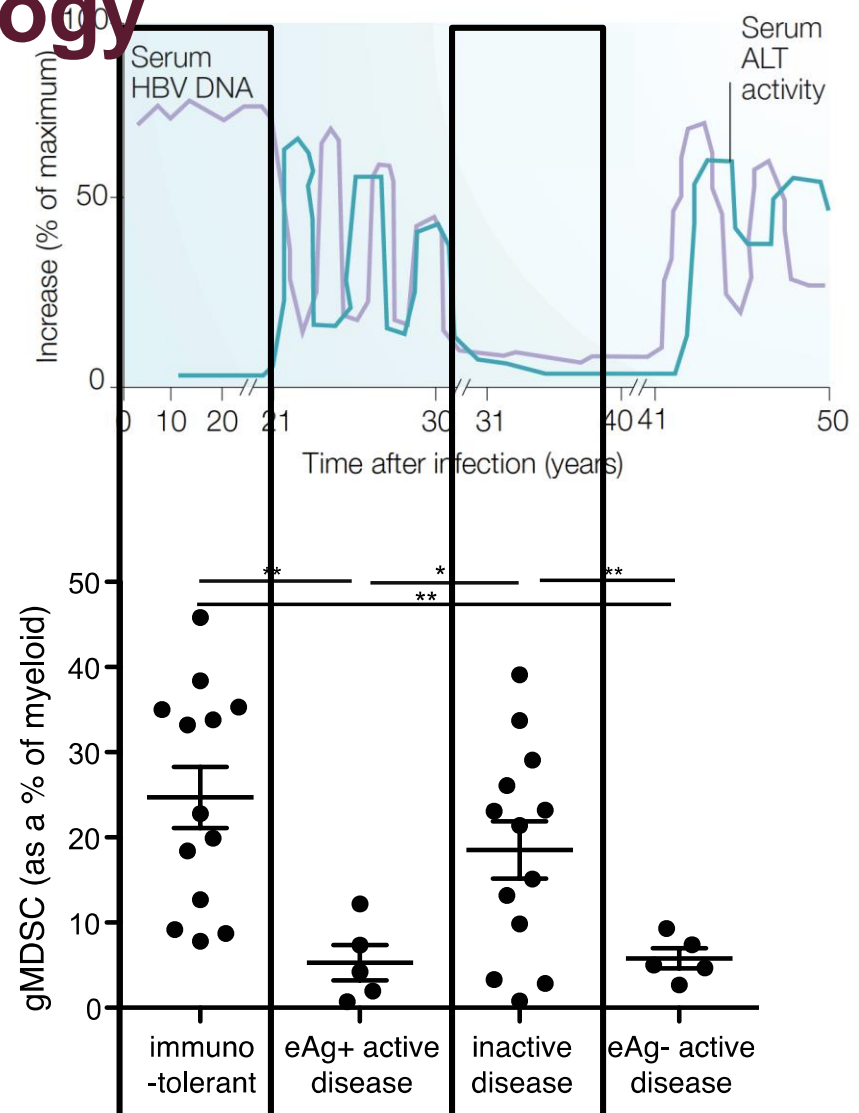
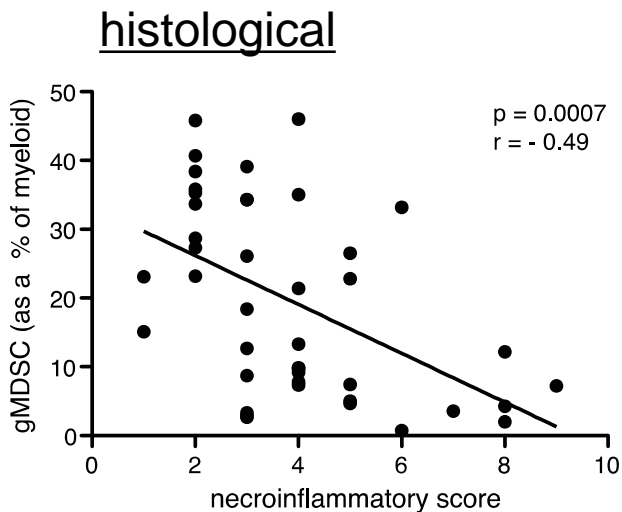
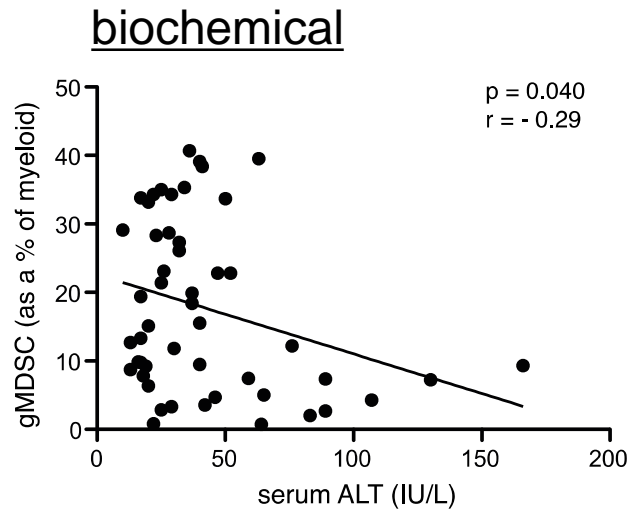




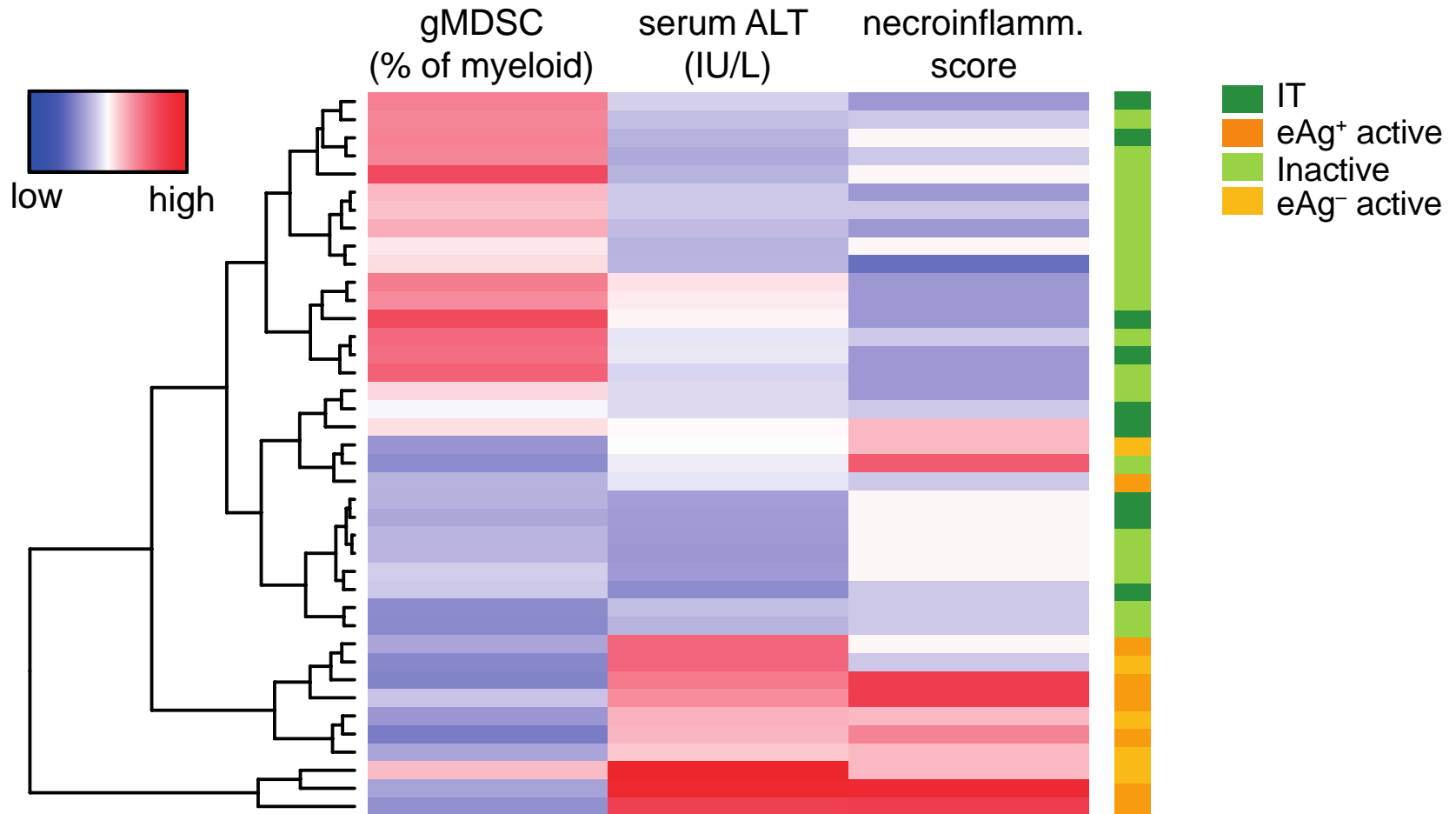
# gMDSC expand in patients replicating HBV without immunopathology



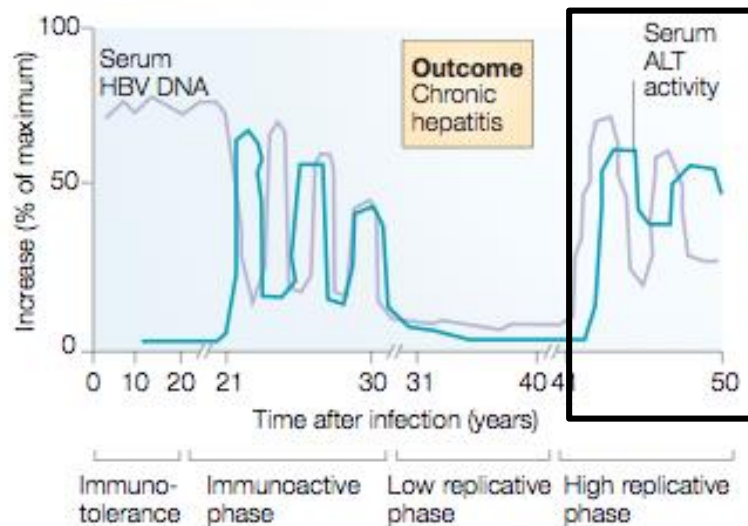
# gMDSC expand in patients replicating HBV without immunopathology



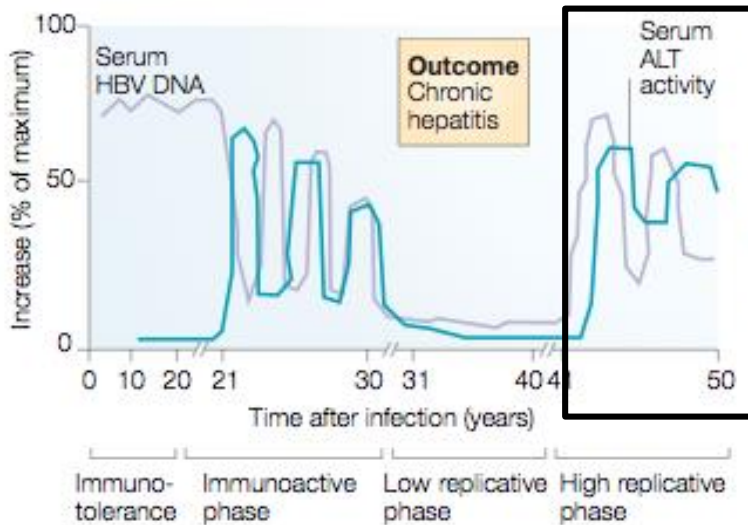
# gMDSC are increased in the absence of liver inflammation



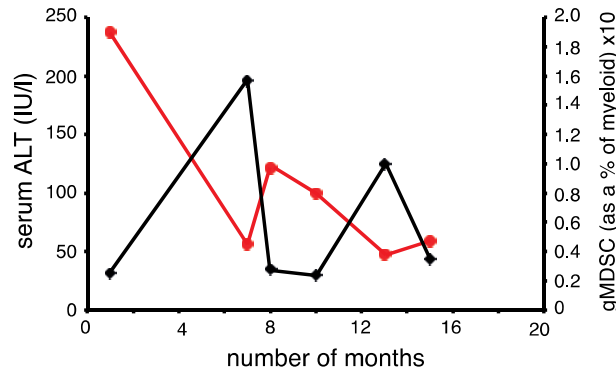
# gMDSC decline before the onset of hepatic flares in eAg- chronic HBV disease



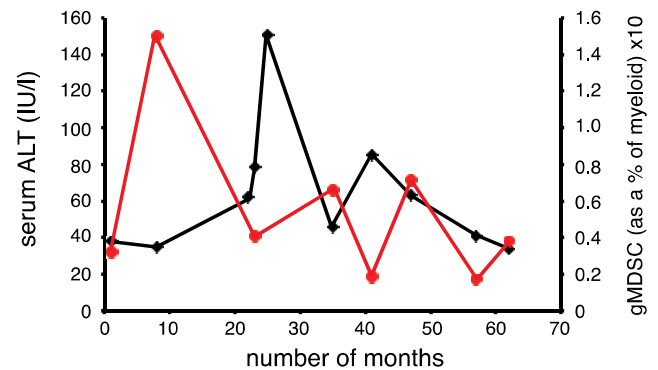
# gMDSC decline before the onset of hepatic flares in eAg- chronic HBV disease



patient C1



patient C2



***Ex vivo* data point to a role for gMDSC in suppressing liver inflammation**

**How could they achieve this?**



# gMDSC drive nutrient deprivation

ARTICLE

## Functional skewing of the global CD8 T cell population in chronic hepatitis B virus infection

Published August 11, 2008

JEM

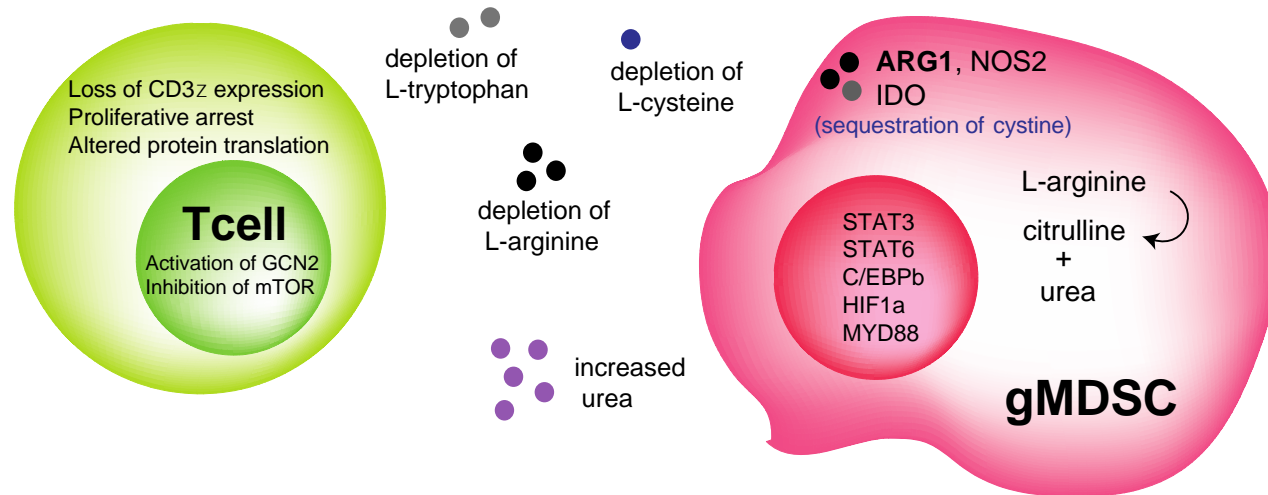
Abhishek Das,<sup>1</sup> Matthew Hoare,<sup>4</sup> Nathan Davies,<sup>2</sup> A. Ross Lopes,<sup>1</sup> Claire Dunn,<sup>1</sup> Patrick T.F. Kennedy,<sup>2</sup> Graeme Alexander,<sup>4</sup> Helene Finney,<sup>5</sup> Alistair Lawson,<sup>5</sup> Fiona J. Plunkett,<sup>1</sup> Antonio Bertolotti,<sup>2,6</sup> Arne N. Akbar,<sup>1</sup> and Mala K. Maini<sup>1,3</sup>

### Global metabolic defect in HBV T cells

- CD3 $\zeta$  downregulation
- Dysregulation in functionality

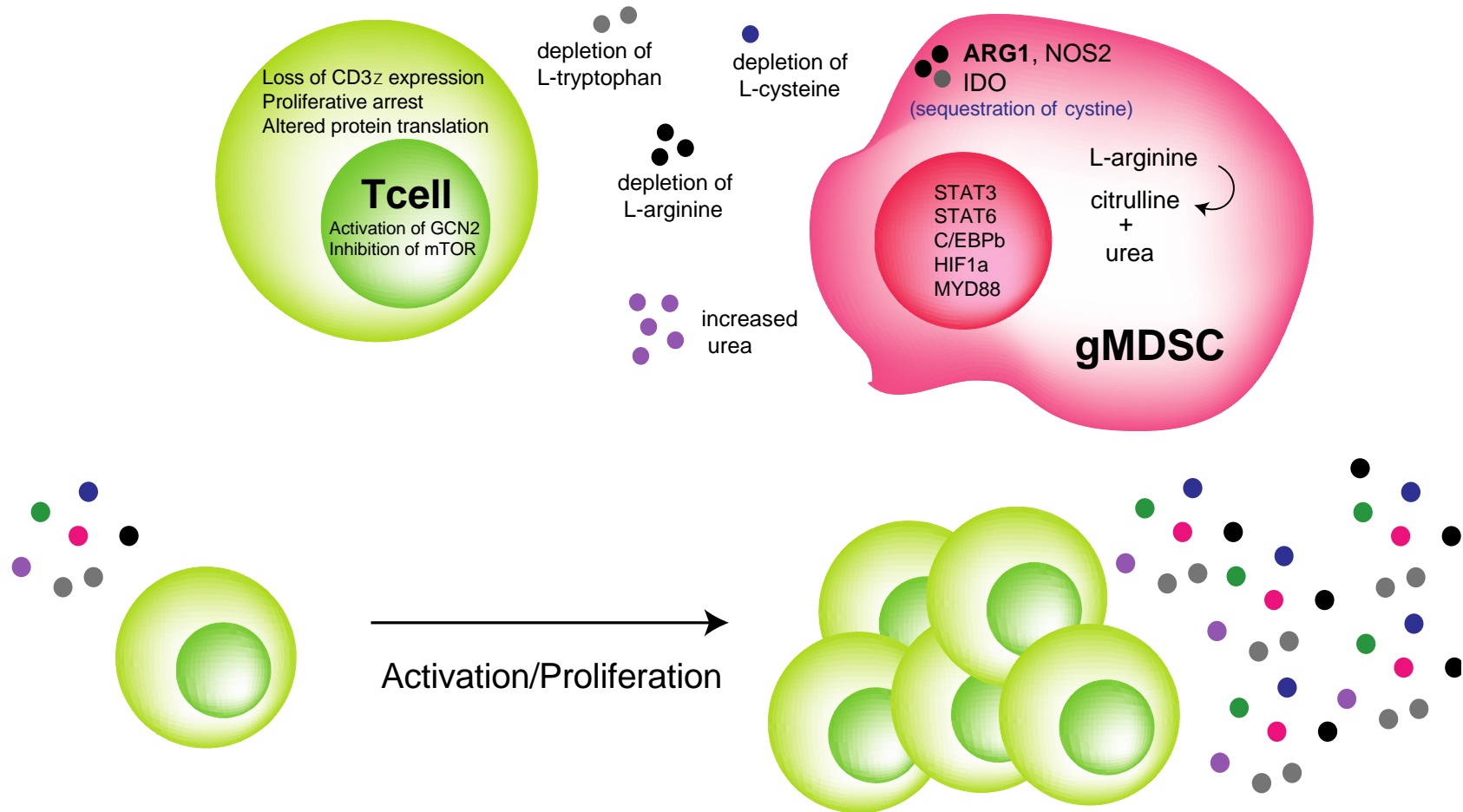
# gMDSC drive nutrient deprivation

## Nutrient deprivation



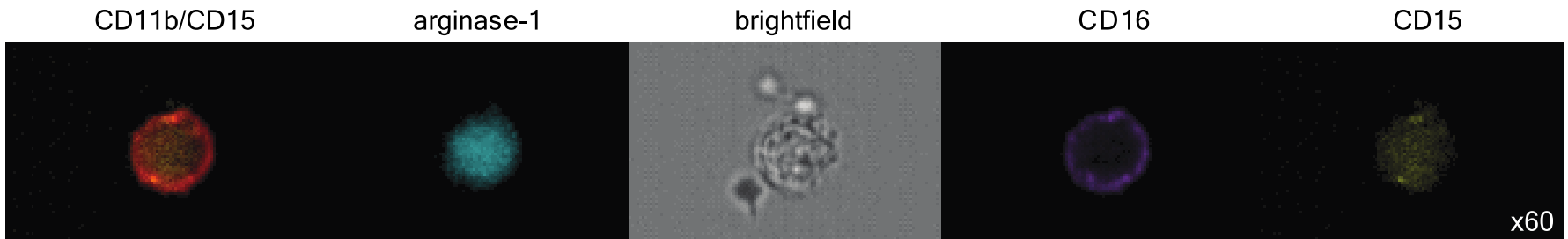
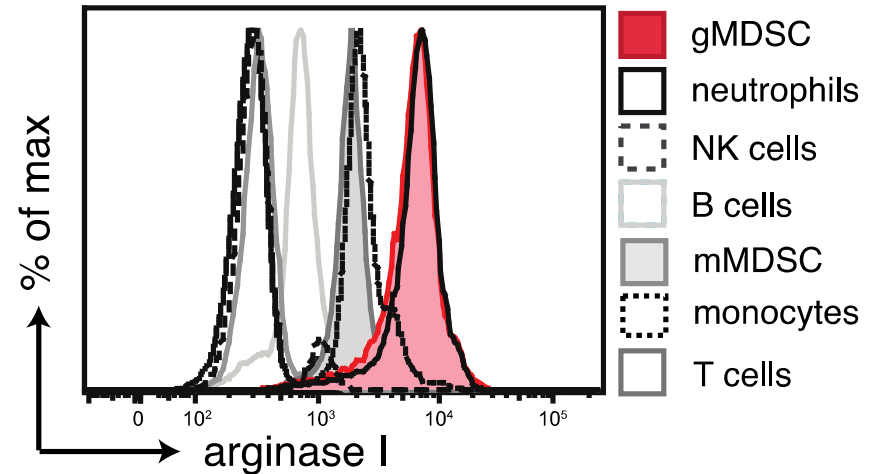
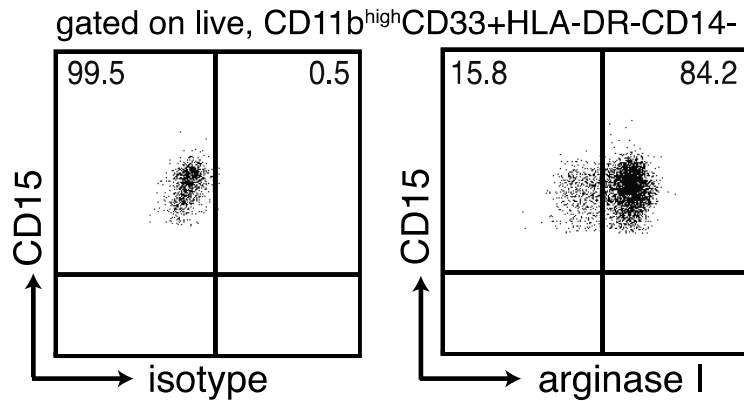
# gMDSC drive nutrient deprivation

## Nutrient deprivation

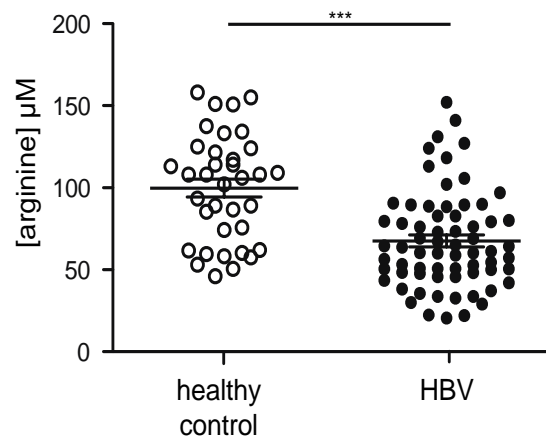
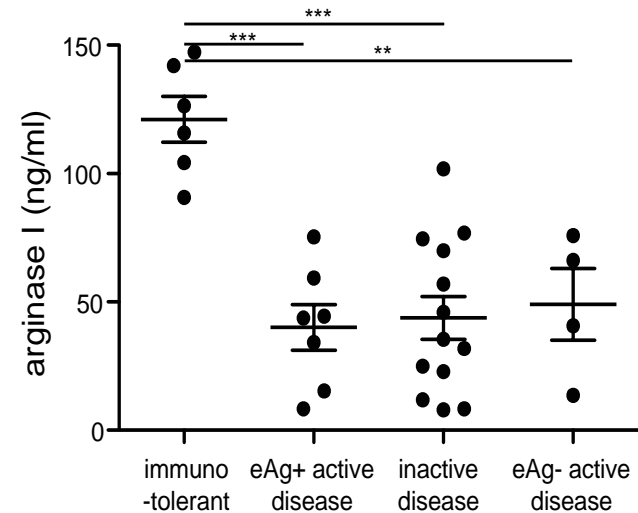
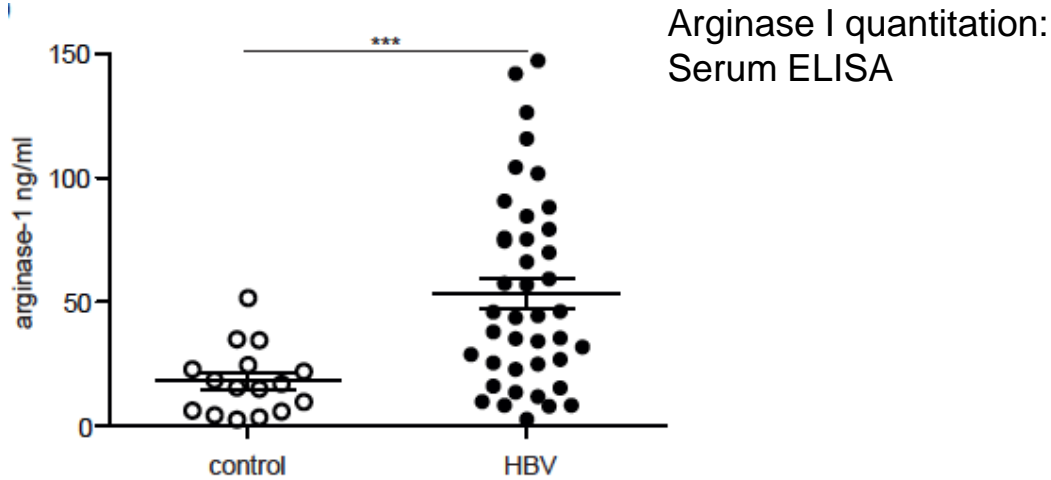


Proliferating T cells require extra amino acids as well as glucose

# gMDSC produces arginase I which depletes L-arginine



# Arginase I is increased and L-arginine is decreased in the serum



L-Arginine quantitation: tandem high-pressure liquid chromatography mass-spectrometry

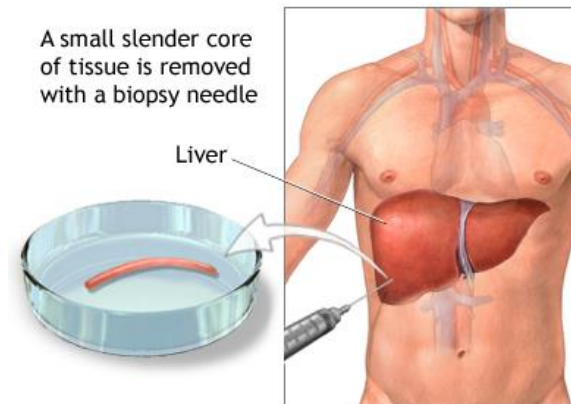
**Do gMDSC reach the liver, the site of HBV infection and pathology?**



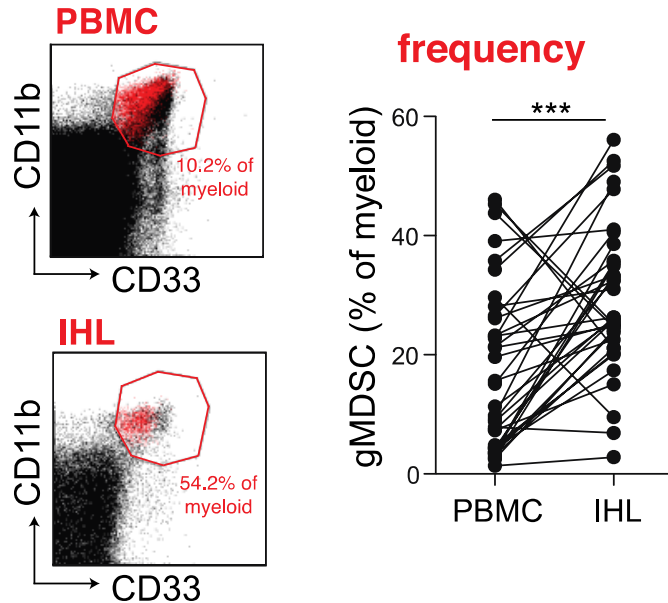
# Do gMDSC reach the liver, the site of HBV infection and pathology?



Upkar Gill  
Patrick Kennedy  
QMUL: Barts & the London



# gMDSC are further expanded in the intrahepatic compartment in HBV

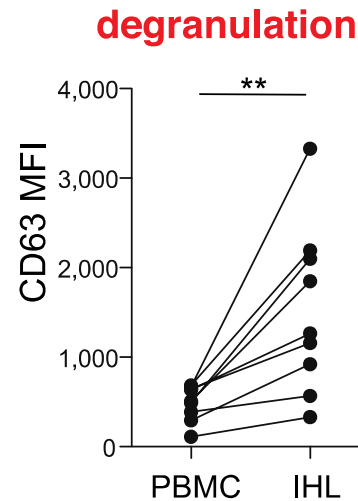
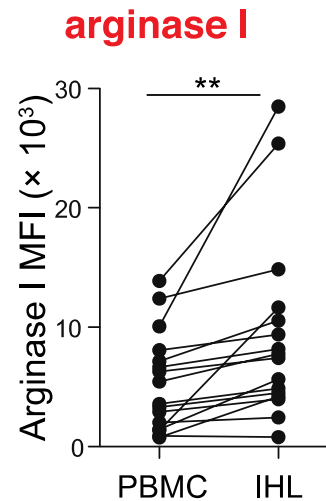
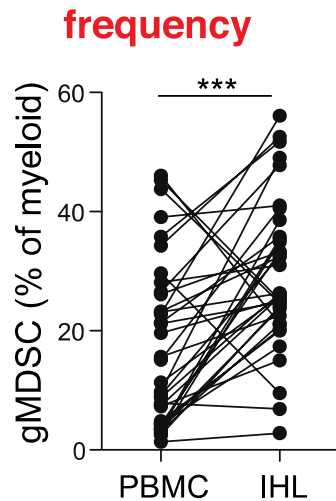
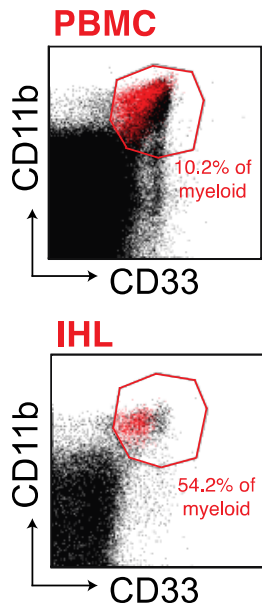


IHL - intrahepatic leukocytes

\*\*  $p = <0.01$

\*\*\*  $p = <0.001$  (Wilcoxon signed-rank test)

# gMDSC are further expanded in the intrahepatic compartment in HBV



IHL - intrahepatic leukocytes

\*\*  $p = <0.01$

\*\*\*  $p = <0.001$  (Wilcoxon signed-rank test)

**What factors promote gMDSC expansion in the liver?**

# pHSC cells promote enhanced gMDSC proliferation/survival

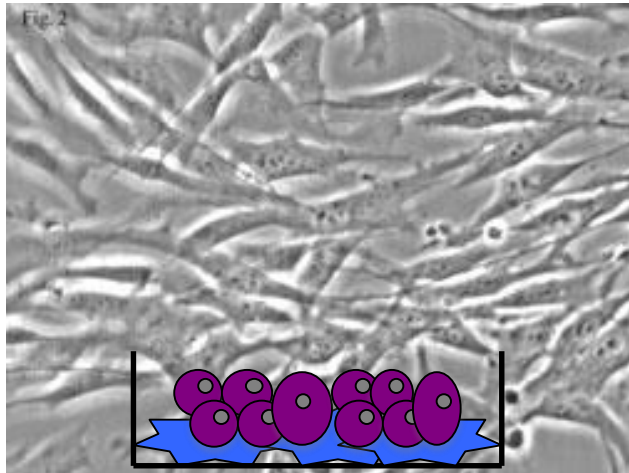
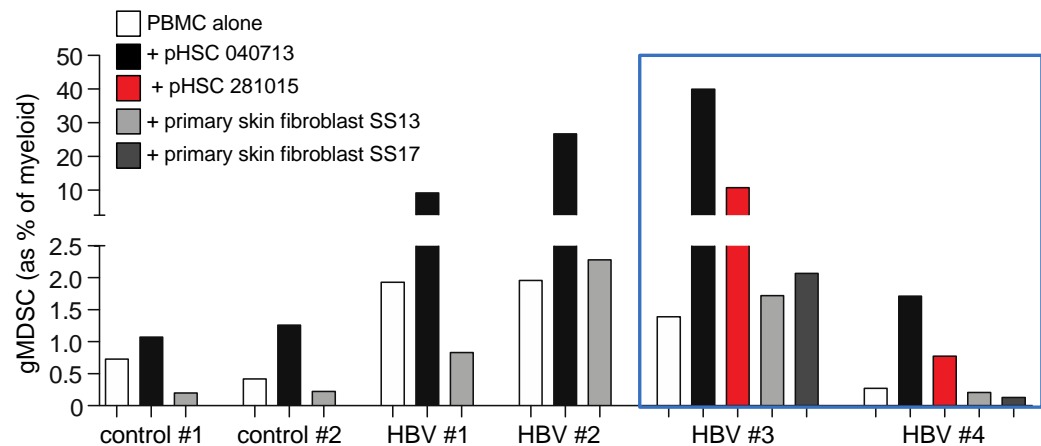
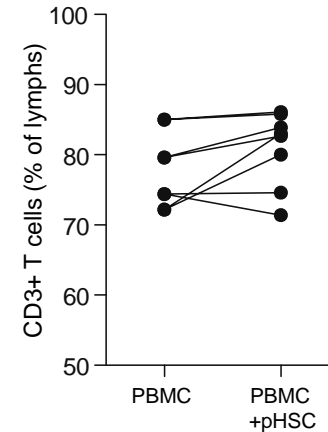
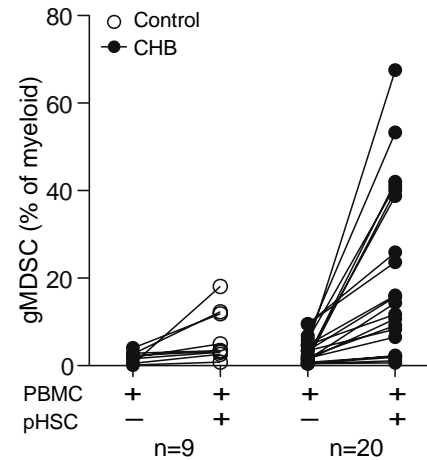
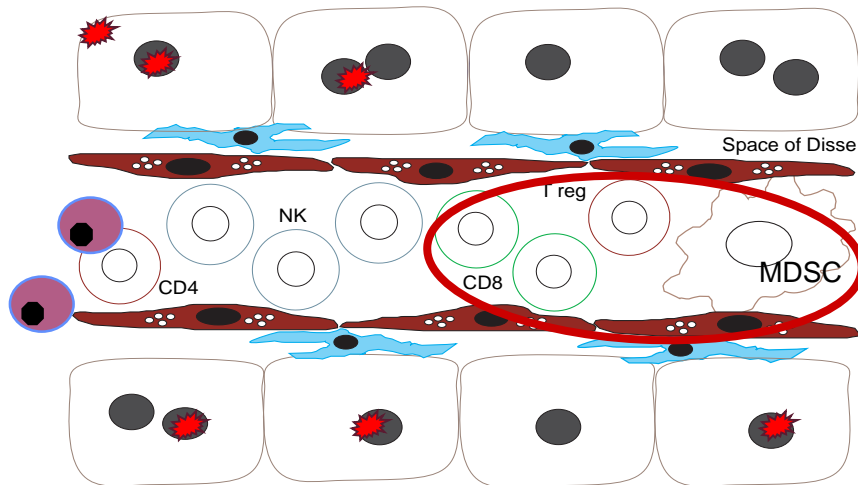


Image from of H. Singh



thanks to K. Singh/ H. Singh/ E.S. Chambers: pHSC/skin fibroblasts isolation

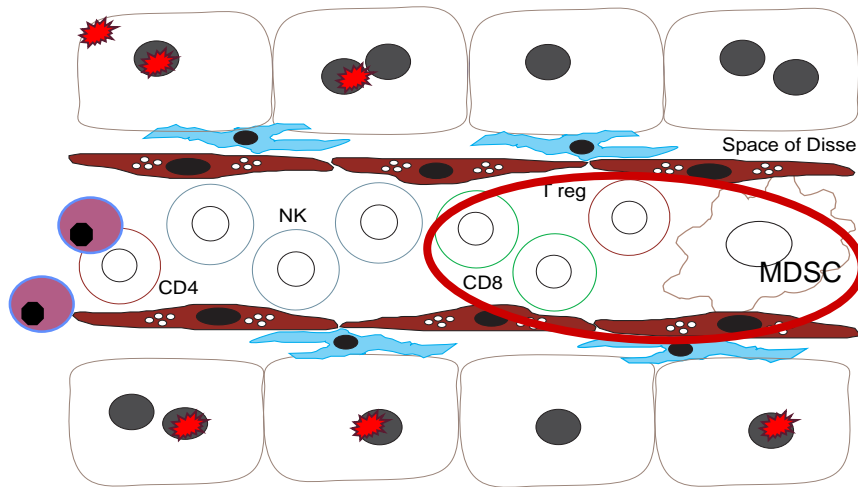
# Can arginase I+ gMDSC suppress T cell immunopathology in the liver?



- HBV is a non-cytopathic virus
- Liver damage: Initiated by HBV-specific CTL, amplified by bystander T cells

Maini et al, JEM 2000  
 Kakimi et al JEM 2001  
 Sitia et al, PNAS 2002

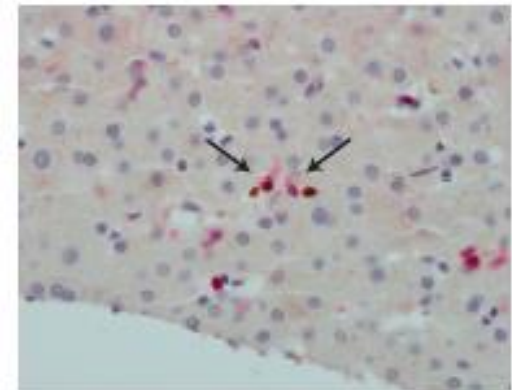
# Can arginase I+ gMDSC suppress T cell immunopathology in the liver?



- HBV is a non-cytopathic virus
- Liver damage: Initiated by HBV-specific CTL, amplified by bystander T cells

Anatomic localisation of hepatic gMDSC?

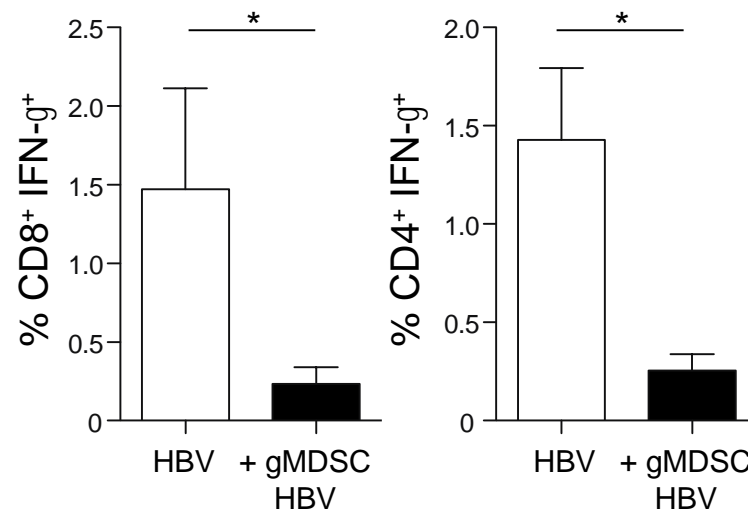
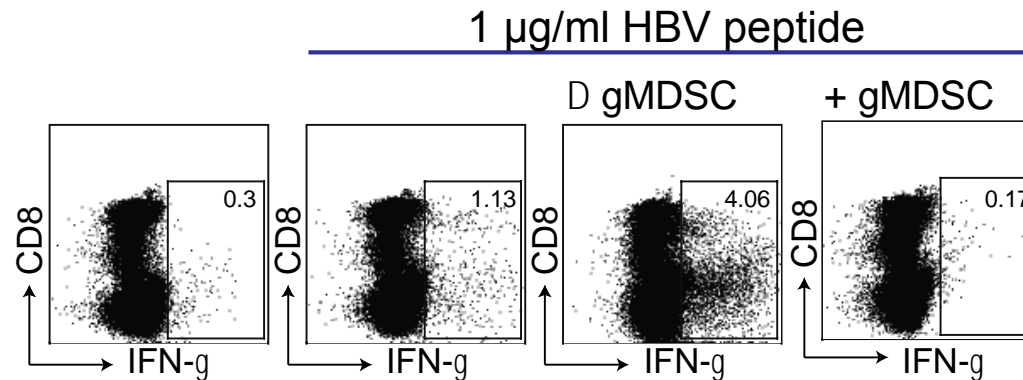
*Visualisation of gMDSC in contact with T cells in liver vasculature*



CD3 red, CD66b brown

Immunohistochemistry: A. Quaglia

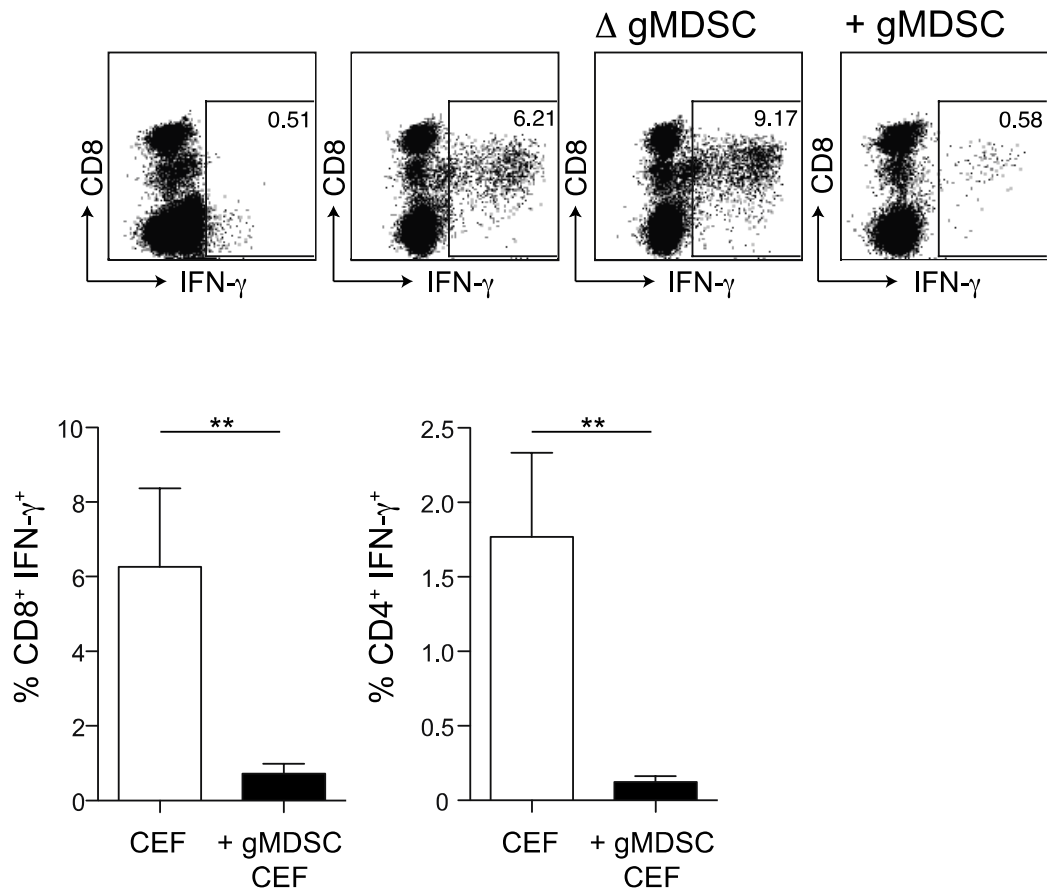
# gMDSC potently suppress expansion of HBV-specific T cells





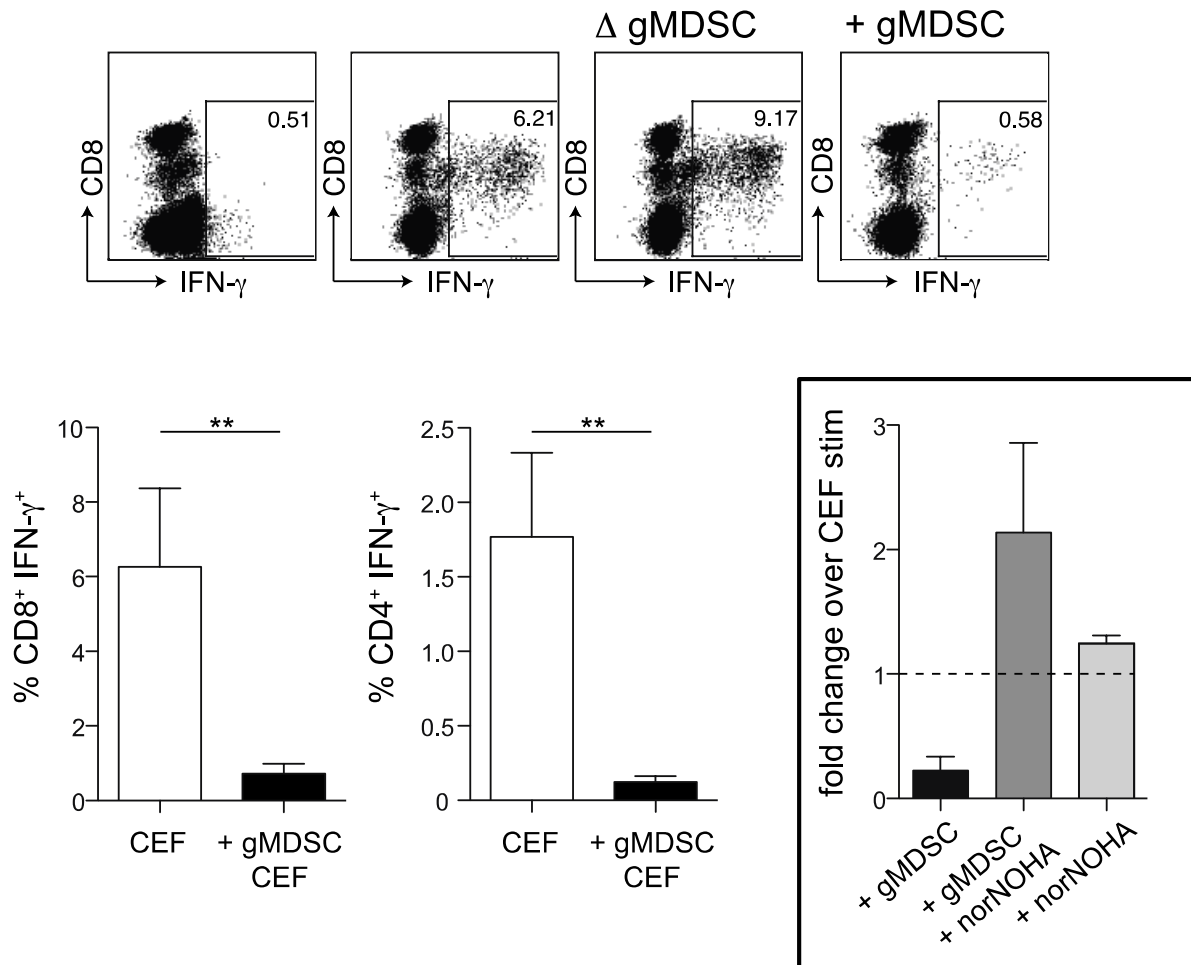
# gMDSC suppress bystander T cells in a partially L-arginine dependent manner

0.5  $\mu\text{g/ml}$  CEF peptide: CMV/EBA/Flu



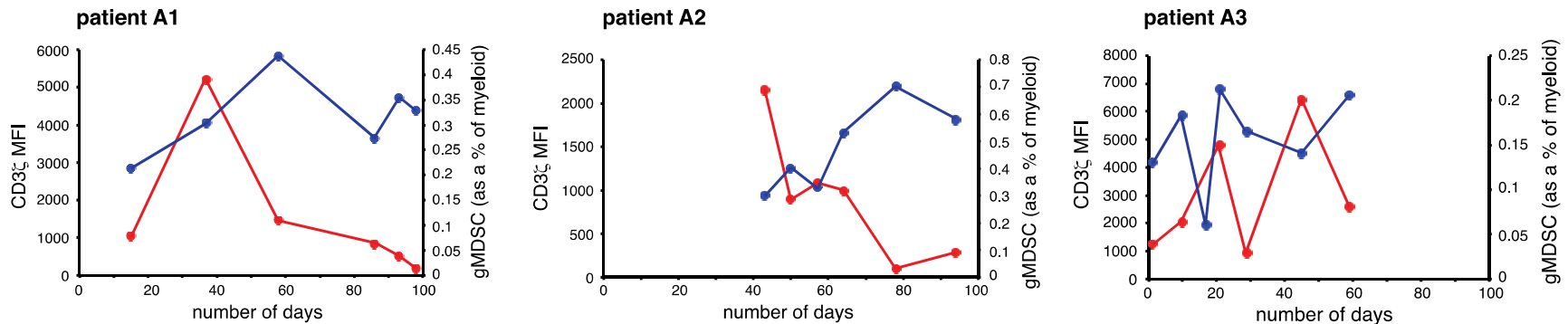
# gMDSC suppress bystander T cells in a partially L-arginine dependent manner

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# Ex vivo: T cells in HBV bare the hallmark of L-arginine deprivation

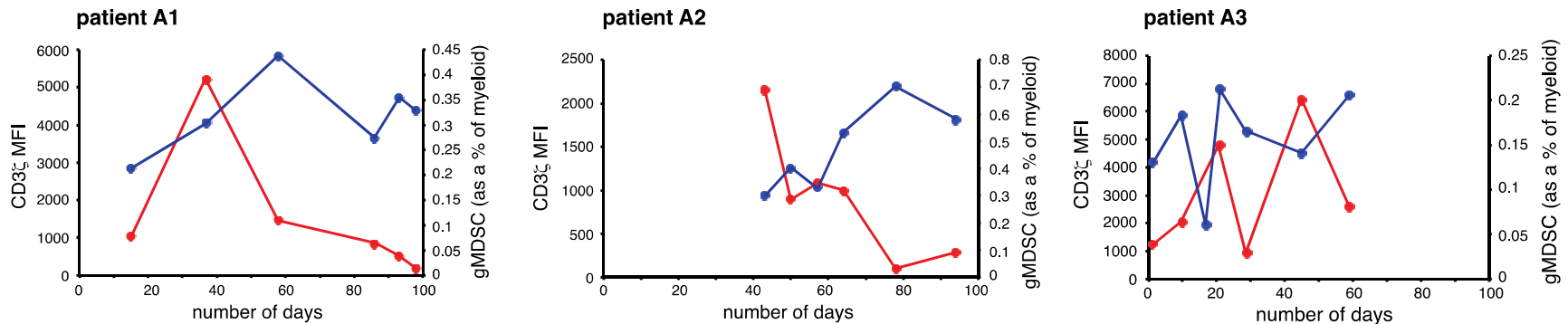
acute, resolving HBV



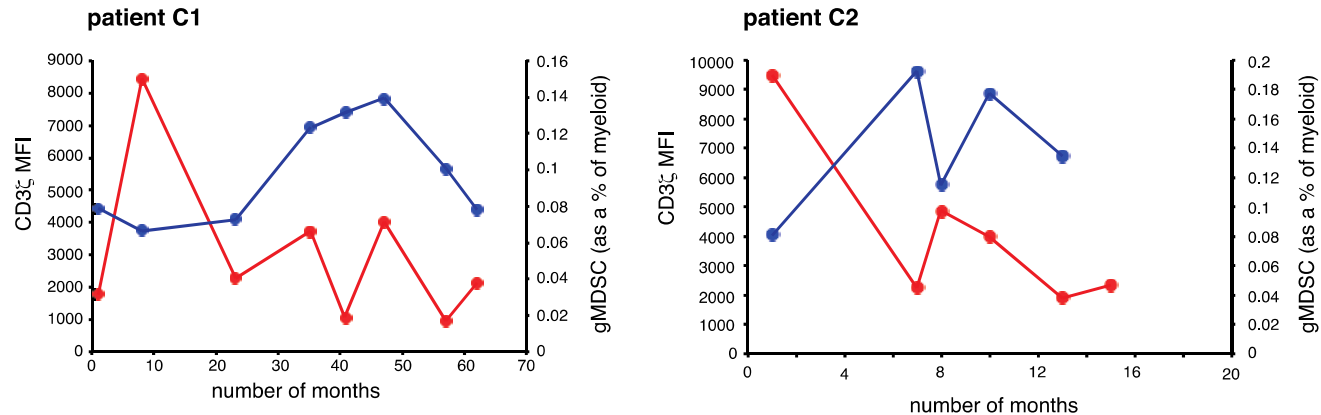
— CD3 $\zeta$  expression MFI on total T cells  
 — frequency of circulating gMDSC (as % of myeloid)

# Ex vivo: T cells in HBV bare the hallmark of L-arginine deprivation

acute, resolving HBV

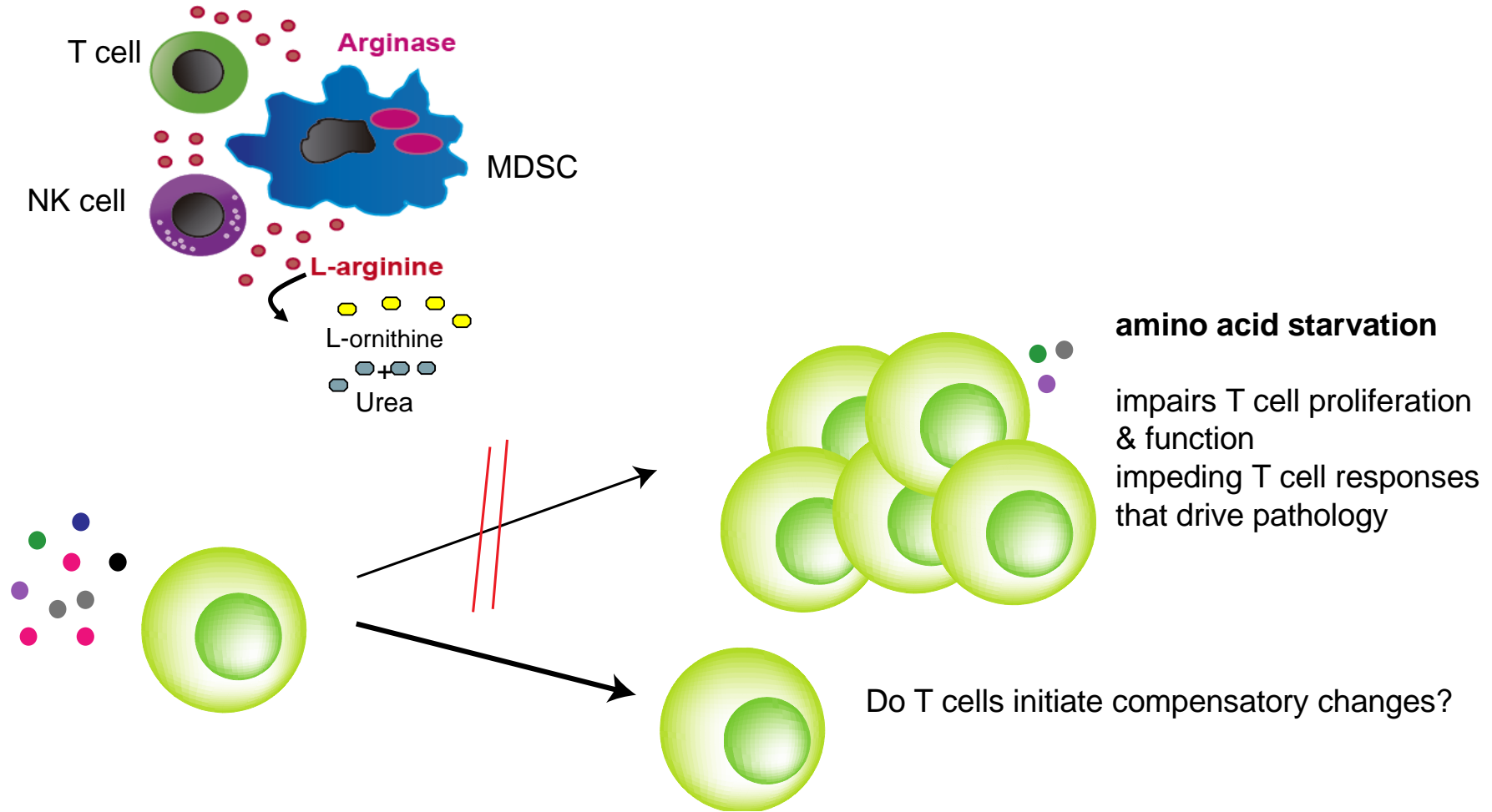


chronic HBV



— CD3 $\zeta$  expression MFI on total T cells  
 — frequency of circulating gMDSC (as % of myeloid)

# Metabolic regulation in HBV at the T cell level

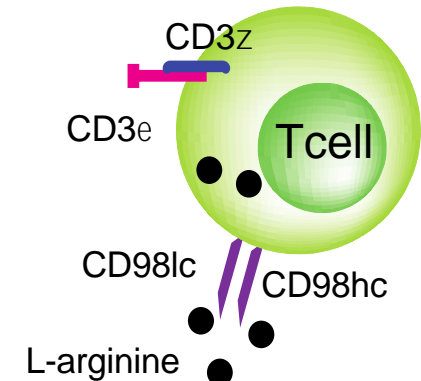


# System-L amino acid transporters: critical checkpoint controlling T cell metabolism

**Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation**

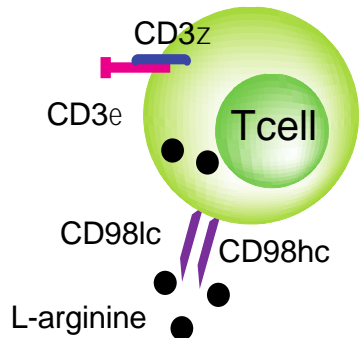
Linda V Sinclair<sup>1</sup>, Julia Rolf<sup>1</sup>, Elizabeth Emslie<sup>1</sup>, Yun-Bo Shi<sup>2</sup>, Peter M Taylor<sup>1</sup> & Doreen A Cantrell<sup>1</sup>

*nature  
immunology*  
VOLUME 14 | NUMBER 5 | MAY 2013

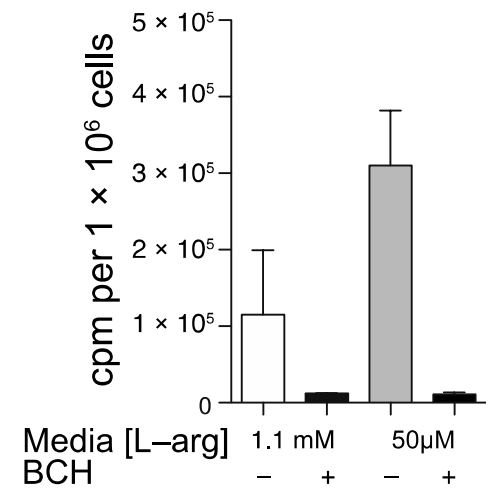
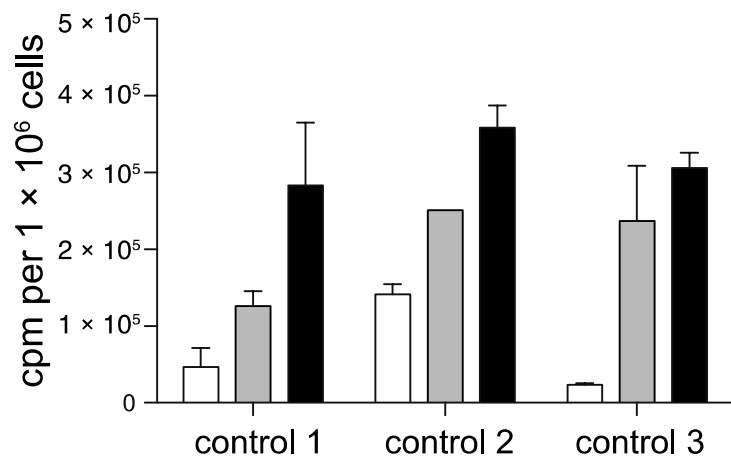
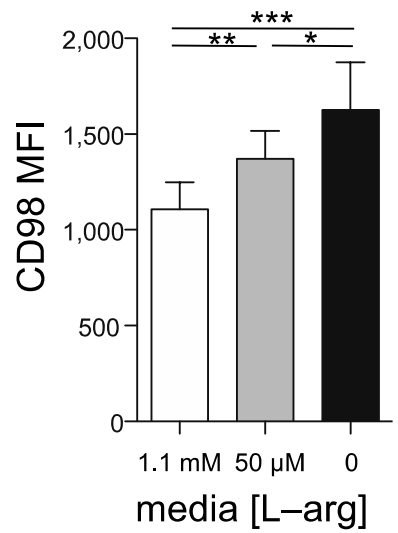


Hypothesis: L-arginine starvation induces an up-regulation of system-L amino acid transporters on T cells

# Compensatory increase in system-L amino acid transport in arginine-starved T cells

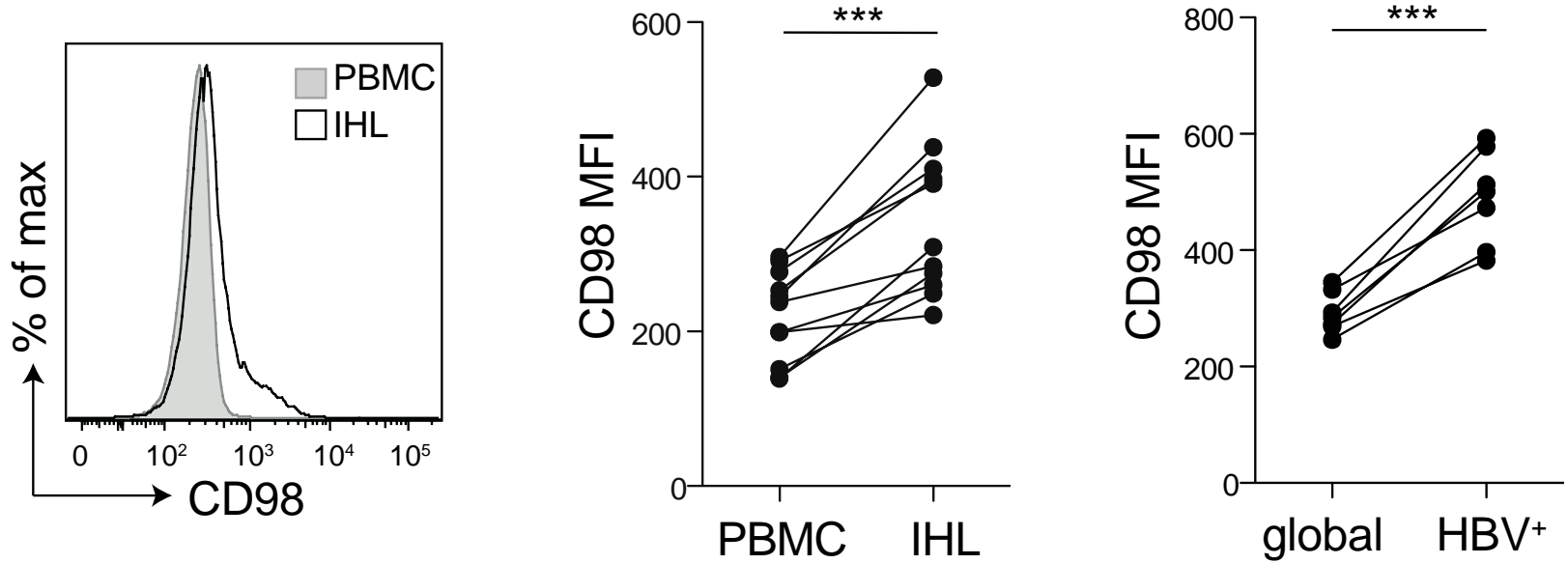


L-arginine free  
 50 $\mu$ M L-arg  
 RPMI 1640 (1.14 mM L-arg)



Pallett et al. *Nat Med* (2015) \* 1.1mM = L-arg in [RPMI]  
 BCH: 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid

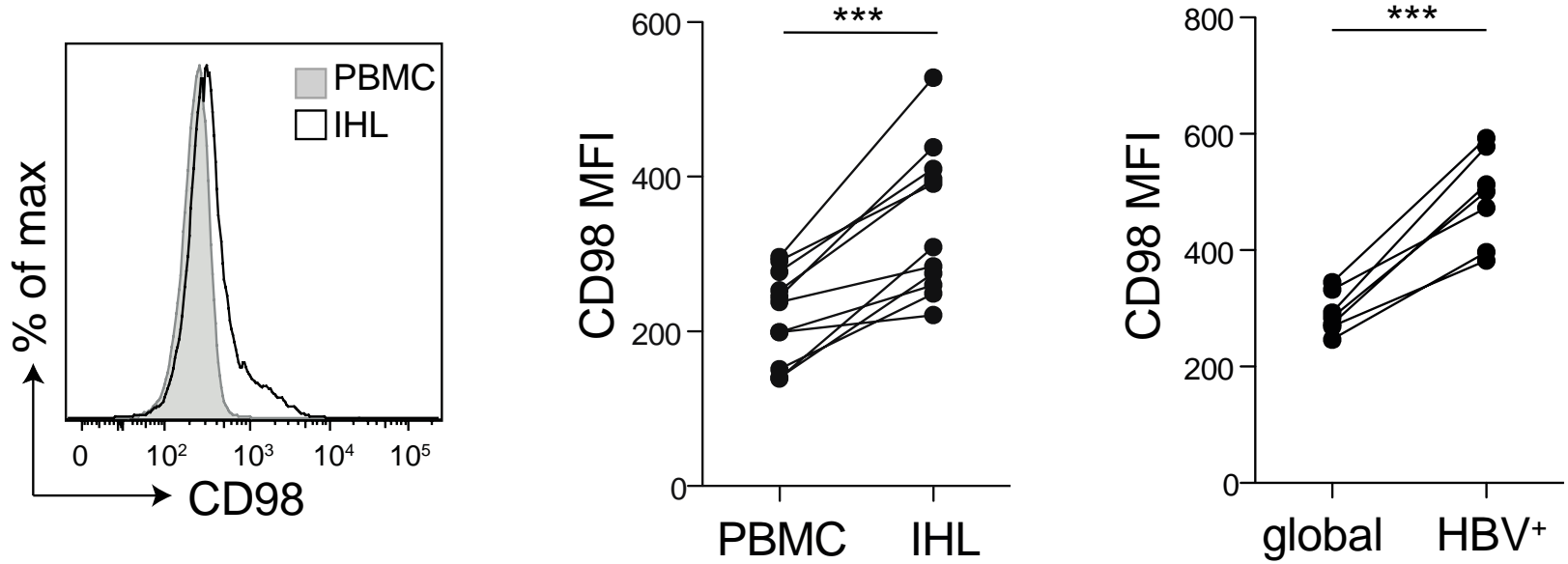
# Intrahepatic and HBV-specific T cells have increase system-L amino acid transporter expression *ex vivo*



\* IHL - intrahepatic leukocytes  
 HBV-specific: HLA-A2-restricted multimer positive



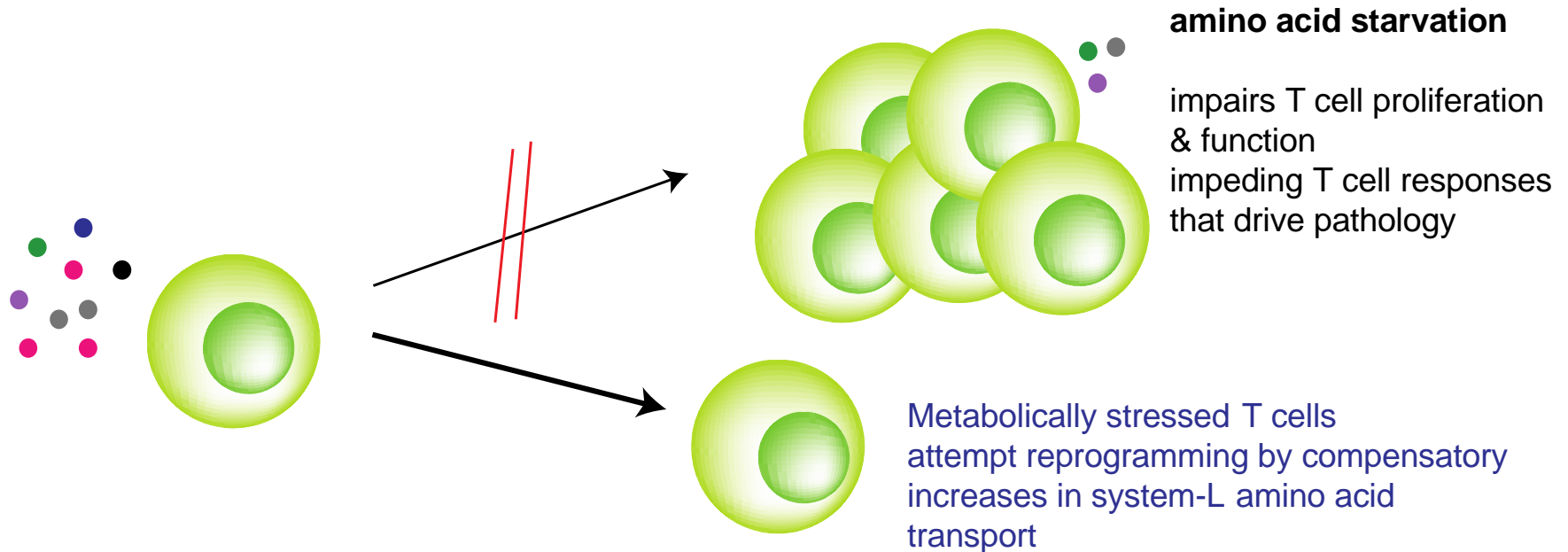
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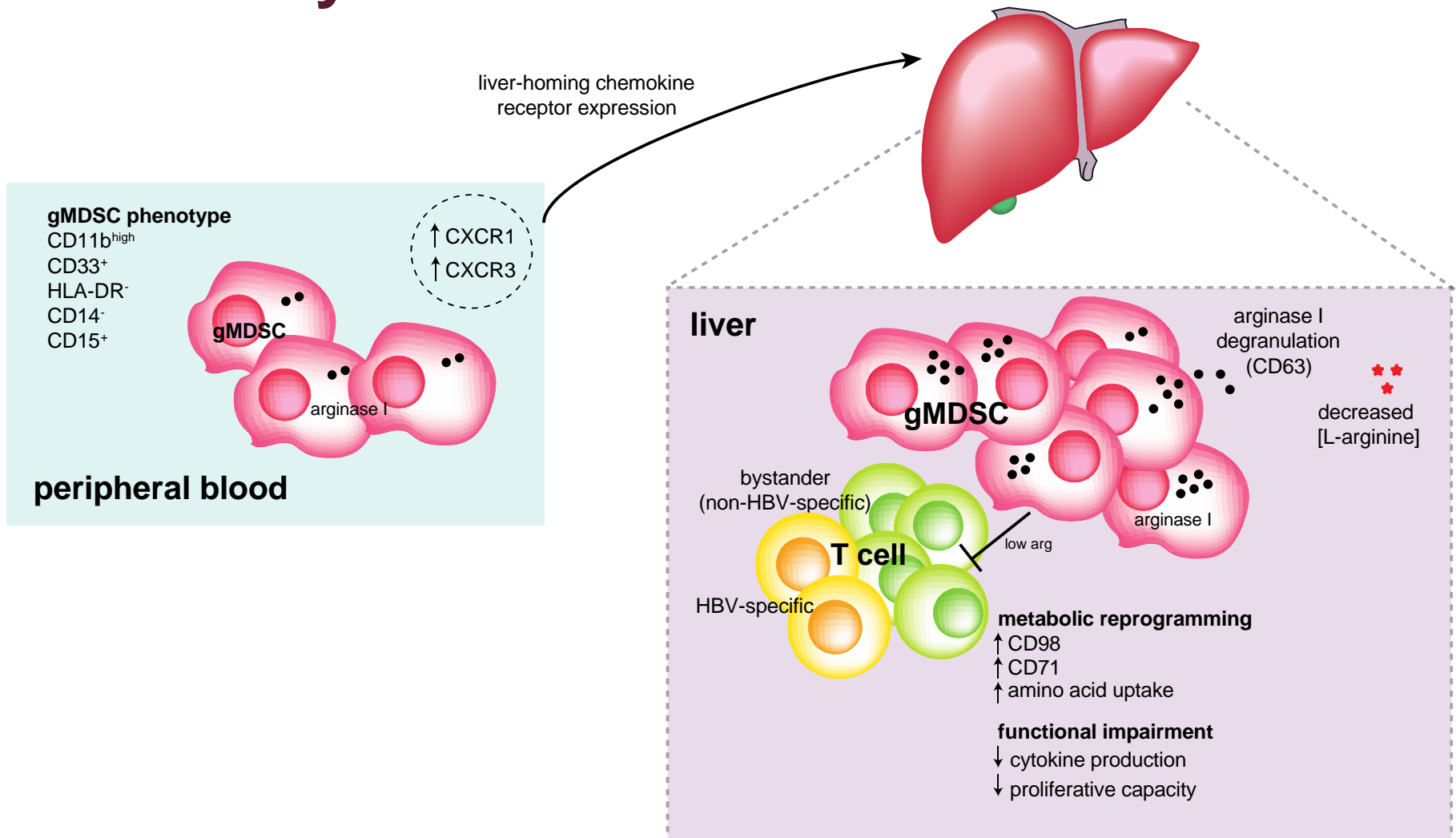
Amino acid transporters calibrate the T cell response to amino acid starvation

# Metabolic regulation of T cells in viral hepatitis



A rheostat & potential therapeutic target to control immunopathology

# Summary



**Mala Maini**

Abhishek Das

Antony Chen

Dimitra Peppas

Jessica Wijngaarden

Nick Easom

Anna Schurich

Kerstin Stegmann

Wei-chen Huang

Itziar Otano

Kasha Singh

Simran Singh

**UCL**

Nathan Davies

Maria Jover-Cobos

Richard Milne

Niclas Thomas

Eleni Nastouli

Emma Chambers

**Kings College London**

Oltin Pop

Alberto Quaglia

**University of Dundee**

Doreen Cantrell

Linda Sinclair



**Royal Free Hospital**

Kito Fusai

William Rosenberg

Francis Robertson

Prof. Davidson

**Royal London Hospital**

Patrick Kennedy

Upkar Gill

**Agency of Science & Technology**

**Research (A\*STAR)**

Antonio Bertolotti

Muzzlifa Haniffa



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